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Volume 39

Edited by

G. C. AINSWORTH and S. D. GARRETT

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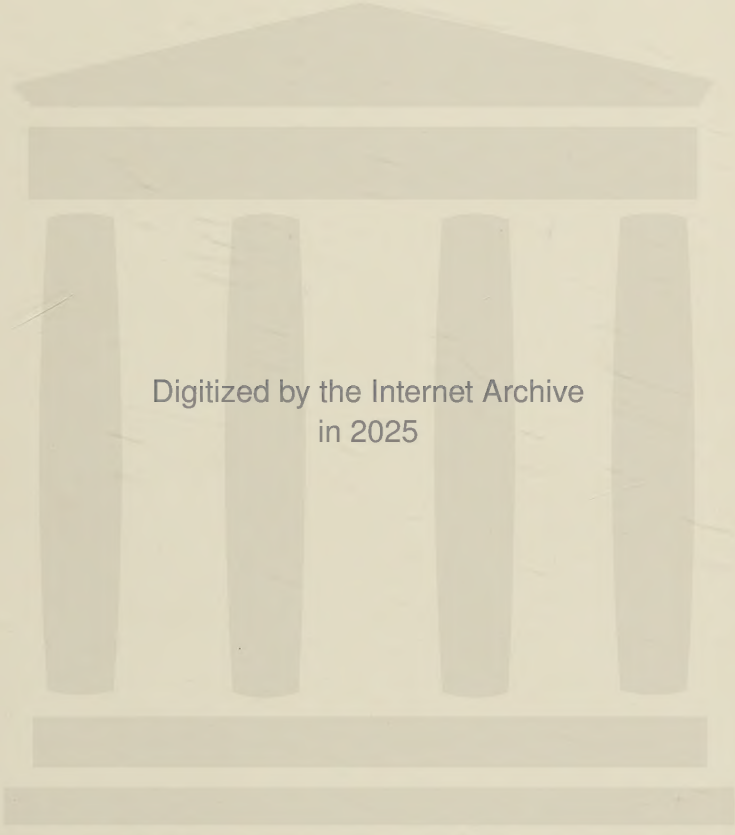

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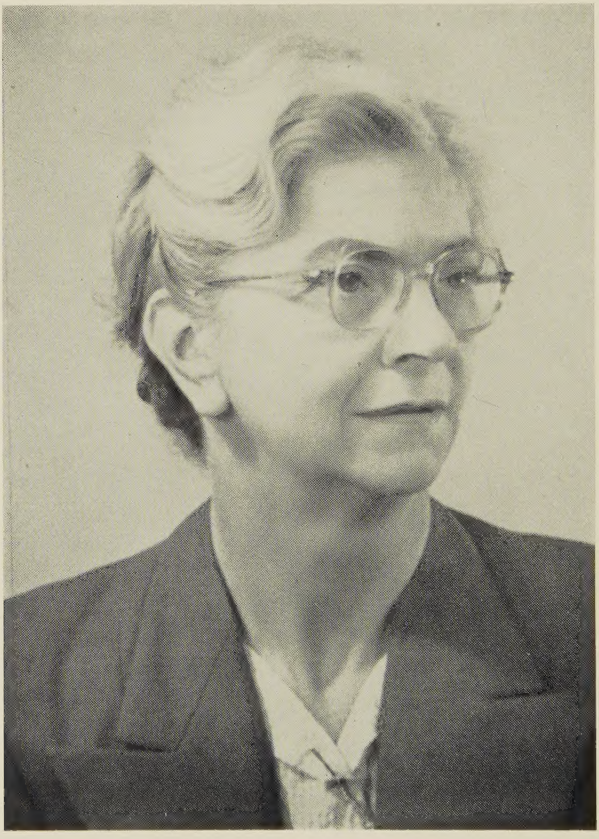

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PRESIDENTIAL ADDRESS

EXPERIMENTAL CONTROL OF FORM AND PHASE IN FUNGI

By LILIAN E. HAWKER

The mycologist is rich in his material. In no other group of living things is there such a wide range of form between the various members of the group, and nowhere is the changing state of the individual or colony more striking or the form of the organism more profoundly influenced by its environment. The protean form of the individual with age or environmental change is at once the despair and the inspiration of the taxonomist, the physiologist and the applied mycologist. To the physiologist, in particular, it is also a challenge. The early mycologists were content to describe and classify their material, but quite soon we find that simple observations of the effects of keeping fungi under varied conditions, or of the effects of weather on their form in the field, are included in otherwise purely descriptive accounts. It is a long road from the contented observation of beauty and variety of form to the translation of these into terms of physics and chemistry. We have far to go to reach that goal. We may never reach it. Nevertheless, in the last fifty years great progress has been made in the interpretation of the effect of environment on form and phase in fungi, and some light has been thrown on the relation between metabolism and morphogenesis.

The practical advantages of a knowledge of the factors controlling the production of sclerotia, rhizomorphs or spores is obvious. The taxonomist must, if possible, obtain spores before he is able to classify his material, while the prevention of the formation of resistant structures or agents of dispersal is important to the plant pathologist and the industrial mycologist. The physiologist is not greatly concerned with the application of his results, but he is vitally interested in the mechanism of the organism. The effects of environmental factors are the tools with which he hopes to explore and dissect this mechanism.

Form

The potential form of a fungus, whether growing in its natural habitat or in artificial culture, is limited and controlled by its genetic constitution. The expression of these potentialities depends on the environment, notably the chemical and physical nature of the substrate and such factors as water supply, aeration, temperature and sometimes light. By the manipulation of these the physiologist can to a large extent alter the form and phase of a fungus, and at the same time may learn much of the internal forces which ultimately control morphogenesis.

When a plate or flask of suitable medium is inoculated with a particular fungus there is usually a period of purely mycelial growth of a more or less

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generally favourable to the production of mycelium. A useful experimental approach is here to try the effects of increasing the concentration of the medium as a whole above that permitting hyphal growth, or to increase the amount of a particular foodstuff relative to the remainder. Thus the addition of vitamins, such as thiamin or biotin, to the medium on which fungi which have only limited ability to synthesize these are growing, may result in greatly increased growth and the production of mycelial aggregates, such as sclerotia, or hyphal strands. As Garrett (1953) and Townsend (1952) have shown, the position is not invariably so simple, and it is always dangerous to attempt to fit all examples into one scheme.

When we consider the control of spore production by the environment we are faced with an even more complex problem. As is well known, fungi produce many types of spores, some of which are produced singly on the mycelium, while others are borne on or in complex sporophores or fruit-bodies. As might be expected, the factors favouring sporulation differ widely with different types of spore. In particular those favouring the production of asexual spores are distinct from those inducing the development of the characteristic sexual or 'perfect' stages.

Asexual sporulation

Any survey of the physiology of sporulation must begin with the pioneer work of Klebs (1898, 1899, 1900). His most striking results were obtained with the Saprolegniales, where he was able to show that certain strains would remain in the vegetative condition indefinitely if kept supplied with fresh nutrients, but that when transferred to water they produced sporangia within a few days. Sexual reproduction occurred when the medium in which these crude cultures were growing was left unchanged. Klebs thus laid the foundations of the experimental approach to the study of morphogenesis, but he made no attempt to analyse his results or to determine precisely the factors operating to produce them or the exact stage which was susceptible to environmental influences.

The production of asexual spores may be taken to be a particular expression of hyphal growth. The threshold requirements for the initiation and development of asexual sporangiospores or conidia often differ quantitatively rather than qualitatively, from those sufficing for mycelial growth. This is particularly true of food requirements. For example, it is now well known, thanks to the work of Steinberg (1919, 1939) and others, that *Aspergillus niger* grows, but is unable to produce conidia, when certain trace elements are at a very low level. Conidia which develop at slightly higher concentrations of copper are lacking in pigment and, as the concentration is increased, not only is the number of conidia increased too, but the spores themselves develop pigment which ranges from yellow, through brown, to the typical black colour when an adequate concentration is reached. The dependence of conidial development in many plant pathogens on a relative humidity greater than that sufficing for mycelial growth is well known. Other factors also have a quantitative effect on the production of either mycelium or asexual spores. That the factors controlling asexual reproduction are not only quantitatively different from those

favouring hyphal growth, but may often differ qualitatively as well, is shown by those fungi which produce more than one type of asexual spore. The work of Bachmann (1895) with *Thamnidium elegans*, of Goldring (1936) with *Blakeslea trispora* and of Barnett & Lilly (1950, 1955) with *Choanephora cucurbitarum* illustrates this point. The production of conidia or sporangia by *Choanephora* depends upon a complex of factors, such as, duration and quality of illumination, relative humidity, and temperature, while quantity and quality of food profoundly influences the proportion of sporangia to sporangiola produced by *Blakeslea* and *Thamnidium*. The actual form of the asexual spores themselves, or of the hyphae bearing them, may also be profoundly altered by changes in external factors, as with the effect of humidity on the length of conidia of *Ramularia vallisumbrosae* (Gregory, 1939).

Sexual sporulation

Biologically the most interesting change in the form of a fungus is that taking place with the development of the perfect stage, and it is here that my own chief interests lie. I need not stress to my present audience the great variety of spore types covered by that most unsatisfactory term, 'the perfect stage'. The so-called perfect or secondary stage includes all those examples among the Lower Fungi where true sexuality is still to be seen, and also those degenerate forms among the Higher Fungi where a nuclear fusion and a subsequent reduction division are the sole remnants of sexuality. The initiation of this perfect stage is the most profound change that takes place in the life history of any fungus. In the Lower Fungi it usually involves a true sexual conjugation or fertilization, leading to the production of a spore differing morphologically and physiologically from the asexual ones. Even in the chytrids, where the conjugating gametes are often indistinguishable from the zoospores, the resulting spore is distinct. In the Oomycetes and Zygomycetes the oospores and zygospores are not only morphologically entirely different from the asexual spores, but they are usually resting spores, distinguished by the nature of the cell contents, by the wall structure and by failure to germinate except under certain restricted conditions. With the Hemi-Ascomycetes the process of ascus formation is comparatively simple, but the ascospores may be endowed with astonishing powers of resistance to adverse conditions, as with *Byssochlamys fulva*, where the heat resistance shown by some, at least, of these spores is great enough to have made this fungus a serious problem in fruit preservation. When we consider the Higher Ascomycetes, the problem is complicated by the production from the vegetative hyphae of fruit-bodies of varying degrees of complexity, within which an association of nuclei, derived either from obvious sexual branches or from apparently similar hyphae, leads to the development of dicaryotic ascogenous hyphae from which the asci are produced. Even such a comparatively simple alternation of generations as that of the production of dicaryotic ascogenous hyphae by the association of haploid nuclei is a process which must be the result of an exceedingly complex series of chemical changes. In the Basidiomycetes, as you well know, the alternation of generations is usually more striking and the fundamental difference between the uninucleate and binucleate phases

of a heteroecious rust, or the primary and secondary mycelia of an agaric, is obvious but entirely unexplained. We have a very long way to go before we can determine the exact moment at which this alternation is initiated, and begin to study the environmental changes which control it. We can, however, already draw on a wealth of information as to the factors which determine the production of the sexual spores of the Lower Fungi or the fruit-bodies of the Higher ones. We can also, to some extent, indicate the kind of environmental changes which are likely to lead to their production.

In the time at my disposal I can only give a few examples of experimental studies on sporulation, and then attempt to formulate the general lines of experimental approach along which we may come nearer to solving this most absorbing problem.

It is obvious that, whether we are studying conjugation in *Mucor* or the production of a complex fruit-body by one of the Higher Basidiomycetes, the process is a complex one and takes place in a series of definite stages. One would not expect the factors controlling these to be uniform, in fact the orderly progress from one stage to another must depend upon a series of changes in the immediate environment, or at least, on chemical changes induced within the cells themselves. It is thus of the first importance that experiments should be precisely related to a particular stage, or stages, in development and not, as with many past studies, vaguely to the complex process of fruiting as a whole.

Primitive aquatic Phycomycetes are particularly useful material for study, since they can be grown in liquid culture media, thus making the study of nutrition easier, and they have a relatively simple life history. From the time of Klebs to the present day they have been favourite objects for the investigation of the physiology of sporulation. Earlier work was confined to observations of cause and effect with little or no attempt at interpretation. Recent work has advanced towards an interpretation of the process of spore production.

Two outstanding examples are Raper's experiments with the dioecious species *Achlya ambisexualis* and *A. bisexualis* (summarized by Raper, 1952) and Cantino's study of *Blastocladiella emersonii* (1951–4).

Raper first defined the various stages in the formation of antheridia and oogonia of *Achlya*. He then showed by a variety of methods, including the growing of male and female thalli in alternate micro-aquaria, that the whole process is controlled by hormones produced by the fungus itself in orderly succession. Thus the process is initiated by the effect on the male thallus of a group of hormones (hormone A), some of which are secreted by the female thallus and some by the male thallus itself. These control the formation of antheridial initials, which is followed by the secretion of another hormone (B) by the male thallus leading to the initiation of oogonial initials by the female thallus. Hormone C, secreted by the female thallus at this stage, causes the attraction of the antheridial branches towards the developing oogonia and the delimitation of the antheridia by the formation of cross-walls on contact with the oogonia. The latter then also become cut off by a cross-wall as a result of the secretion by the antheridia of yet another hormone (D). Raper considers that fertilization itself is also under

hormonal control, but at present the difficulty of demonstrating this has not been overcome. The difficulty of obtaining these hormones in adequate quantity has so far prevented their identification, but the action of Hormone A can be reproduced by certain organic acids, of which glutaric acid is the most effective. Raper showed that external factors influenced the intensity of sexual reactions at any stage. By thus analysing the process of oospore production and demonstrating that, at least, the early stages are under hormonal control, Raper has opened the way for exact studies of the effect of environment on particular stages of the process.

Cantino (1951–4) has made use of the aquatic *Blastocladiella emersonii* as the subject of a most interesting study of the relation between metabolism and morphogenesis. It is well known that the members of the Blastocladiales produce both thin-walled sporangia, giving rise to asexual zoospores, and thick-walled resting sporangia, which, in some species, have been shown to produce motile gametes. In *Blastocladiella*, Cantino has shown that the zoospores, on germination, will produce either a thallus bearing the thin-walled sporangia or one bearing the resting ones. The nature of the thallus which a particular spore will produce is determined by the concentration of bicarbonate in the medium. With low concentrations of bicarbonate the thalli tend to produce mostly thin-walled sporangia, but with higher concentrations only resting sporangia are formed. After a certain critical stage in development has been reached it is no longer possible to influence the type of thallus in this way. Cantino suggests that a decrease in permeability prevents both the loss of accumulated bicarbonate from the cell and the entry of this substance from the medium. He correlates the effect of bicarbonate on morphogenesis with its effect on certain enzyme systems. Again in this study, as with that of Raper, we have an attempt to pinpoint the exact stage in development at which a particular factor is operative and to interpret its effects through those on a particular metabolic reaction. Such studies, while intrinsically far more difficult to carry out, are of much greater value than the mere recording of empirical results.

We have a great deal of information on the factors inducing zygospore formation in the Mucorales, but much of this is contradictory, perhaps because the investigators were considering the process as a whole rather than as a series of distinct stages each dependent upon a complex system of syntheses of enzymes and other substances. In the Department of Botany at Bristol we noticed that if a fruiting culture of *Mucor sexualis* was stored at 7° C. or less, the process of conjugation continued and led to the production of mature zygospores if the stage of gametangial delimitation had already been reached before the culture was subjected to low temperature, but that no new conjugations took place unless a large number of mature zygospores were already present on the plate. A detailed study by Perkins (1952) and Roberts (1954) showed that the inhibitory effect of low temperature was indeed limited to the early stages of conjugation. Further work suggests that the low temperature probably acts by inhibiting some synthetic process essential to further development. This is supported by the observation that the presence of a large number of mature zygospores allows the initiation of new gametangia even at low temperatures. At present there is little

indication of the nature of the enzyme or other substance concerned, but the problem is obviously capable of further experimental investigation, which is now being undertaken.

The problem of the physiology of fruit-body formation among the Higher Fungi is obviously even more complicated than that of the formation of sexual spores among the Phycomycetes. The Pyrenomycetes have been favourite objects for investigation since many of them produce their perithecia readily in culture under easily defined conditions. My own studies with *Melanospora* (now *Sordaria*) *destruens* (summarized by Hawker, 1950) were concerned mainly with the numbers of perithecia formed and with the time from inoculation of a plate to that of the appearance of the first recognizable perithecial initials. Later workers, such as Barnett & Lilly (1947, 1947*a*), Lilly & Barnett (1949), working with species of *Sordaria*, *Chaetomium*, etc., have rightly concerned themselves with maturation and pigmentation of ascospores also. In general, a medium with a high concentration of hexose sugar favours mycelial growth of certain Pyrenomycetes but tends to repress, or at least to delay, perithecial development. More complex carbohydrates, provided that the particular fungus can synthesize the appropriate hydrolytic enzymes, tend to reduce the amount of mycelium produced and to induce earlier and greater production of fruit-bodies. With *Sordaria destruens* it has been shown that this is associated with an increase in respiratory activity (Hawker, 1947). A similar correlation between intensity of respiration and the amount of fruiting was demonstrated in experiments on the effect of changes in the amount of thiamin in the medium. It was further shown that the amount of glucose optimal for fruiting increased with increase in the amount of this vitamin. It is now a commonplace that a number of vitamins are essential for growth of all fungi but that some can synthesize them in adequate amount, while others either synthesize an amount of a particular vitamin sufficient for part of their needs, or are unable to synthesize any at all. These require an external supply of the particular vitamin for optimum growth. The amount of these substances necessary for fruiting is usually higher than that sufficing for vegetative growth. Lilly & Barnett (1951) conclude that fruiting of *S. fimicola* and of other Pyrenomycetes studied by them depends on a certain minimum concentration of both thiamin and biotin in the mycelium. They interpreted the unfavourable effects of certain environmental factors, such as low pH of the medium, through their influence on synthesis and utilization of these vitamins.

Recently, Hirsch (1954) and Hirsch & Westergaard (1954) have studied the development of perithecia of *Neurospora crassa* from the small sterile protoperithecia. The latter are produced in monospore cultures, but do not develop further unless conidia of opposite strain have been added to the culture. Even then they do not develop unless environmental conditions are favourable. Hirsch & Westergaard make a detailed comparison of the factors leading to the production of ascospores and to the production of the black melanin-type pigment, and conclude that synthesis of the latter is an important and integral part of the process of spore formation. It would seem to be equally likely that it is a parallel process resulting from the same conditions.

Such analytical studies can go far towards the interpretation of the process of fruit-body formation. Other factors, in addition to that of nutrition must, however, be taken into consideration. The work of Buller (1909–34) has shown the importance of light and gravity in the determination of the form and orientation of fruit-bodies. The tremelloid outgrowths, occasionally seen on the caps of various agarics, have been attributed to the effects of air currents (Keyworth, 1942) and it is well known, that the size and form of the fruit-bodies of many fungi are influenced by light, aeration and humidity. An outstanding contribution is the recent work of Plunkett (1953), which some of us have heard him describe. By exact experimental work, under carefully controlled conditions, he has shown that light, aeration and humidity are all concerned in the elongation of the stipe and the formation and expansion of the pileus of *Collybia velutipes*. He has since extended his studies to include *Polyporellus brumalis* (1954). He has further shown that the environmental factors interact in a complex fashion.

Conclusions

Some general conclusions can be drawn from the available data. I have already pointed out that changes in the form of the mycelium leading to the production of oidia, chlamydospores, sclerotia, etc., are often the results of the increase in concentration of one or more foodstuffs but that other factors may limit or stimulate these processes. Further, the production of asexual spores often, but not always, results from an intensification of the factors, such as food supply, favouring mycelial growth. When we come to the change to the perfect stage, conditions necessary for this are often radically different from those giving maximum vegetative growth. As a general rule the range of temperature, hydrogen-ion concentration of the substrate, humidity, concentration of carbon dioxide, etc., which permit the development of the perfect spore stage are narrower than those allowing the development of vegetative hyphae, or even of asexual spores. This is also true to some extent of nutritional factors. Thus certain fungi, which are able to synthesize sufficient vitamins for the growth of mycelia, need an external supply before fructification takes place. The threshold value of both macro- and micro-nutrients necessary for spore production is almost always higher than that allowing hyphal growth. At the other end of the range fruiting is usually inhibited by much lower concentrations of nutrients than those necessary to inhibit growth. Transfer of a well-nourished mycelium to a more dilute medium often results in fruit-body formation, but mere starvation of a colony usually prevents fructification altogether, as might be expected. The amount of foodstuff required to build the large fruit-bodies of the higher Ascomycetes and Basidiomycetes is obviously large. Various methods have been used to combine an ample food supply with a concentration low enough to permit fruiting. These range from the simple, but effective, method of supplying large amounts of nutrients in a large volume of relatively dilute solution (Plunkett, 1953), to the periodical addition of nutrients in sufficient quantity to maintain the initial level in the medium, or the actual transfer of the mycelial mat to water or dilute media. The favourable effects often produced by the use of

relatively complex carbohydrates are due, in part, to the rate of breakdown being such that an inhibitory level of hexose sugars is never reached. It has been shown by my own studies of *Melanospora destruens* (Hawker, 1947) and by Buston, Jabbar & Etheridge (1953) for *Chaetomium globosum* that the ease with which various carbohydrates become phosphorylated also influences fruiting.

While much has been done by well-designed simple experiments to test such conclusions as these, it is obvious that the fundamental changes within the fungus which lead to visible changes in form and phase cannot be determined by such methods. A more exact analytical approach is essential and, as we have seen, some attempts at such an approach have recently been made. It is by no means clear whether visible changes in growth-form are due to fundamental changes in metabolism or to a speeding up and intensification of metabolic processes. Only exact and painstaking study can decide this. The synthetic powers of different fungi vary so greatly that the underlying essentially uniform pattern of metabolism tends to be masked. Such variations, if viewed in their proper perspective, are invaluable signposts to the course of metabolism. They indicate a possible 'way in' to the problem, which gives the biological chemist a starting-point, but they do not signify an essential difference in metabolism. By studying these 'blocked' synthetic processes in various fungi and in mutants of particular species, much has been learnt, but little attempt has been made, as yet, to apply such methods to the critical phases of morphogenesis. A great step forward has been made by those few investigators who have attempted to confine their observation to definite stages in reproduction instead of to sporulation as a single process. There is no doubt that even the simplest change in form or phase is not one, but a series of changes, each of which is brought about by a particular enzyme complex, and each of which is, accordingly, influenced differently by environmental factors. The investigator must aim at translating the effects of the various external factors into internal changes in the cell itself. It is within the cell that the vital clue to the nature of the mechanisms controlling changes in form and phase must be sought. The recent advances in the technique of the study of cell physiology must not be overlooked by the experimental mycologist. His material, although variable, is in many ways relatively easy to handle. There exists a mass of empirical data on which to build. As I have pointed out, recent workers have attacked the problem from a number of different angles but with the same ultimate object of interpreting the effect of the environment on particular stages of development. We are, I think, beginning a new chapter in the study and interpretation of morphogenesis in fungi, and I am sure that we can look forward with confidence to great progress in the near future. I could not, in the time available, give a complete survey of this immense field, and perhaps I have been too ambitious in my choice of subject, but I have tried to indicate something of the problem as it exists and to suggest the lines along which it may be tackled. If some of you, as a result, are encouraged to tackle it you will certainly find it a most rewarding study.

REFERENCES

Bachmann, J. (1895). Einfluss der äusseren Bedingungen auf die Sporenbildung von *Thamnidium elegans*. *Bot. Z.* **53**, 107–130.

Bail, T. (1857). *Flora*, **40**, 417–430, 433–444; reference in Foster, J. W. (1949), *Chemical activities of fungi*, New York.

Barnett, H. L. & Lilly, V. G. (1947). The effects of biotin upon the formation and development of perithecia, asci and ascospores by *Sordaria fimicola* Ces. and de Not. *Amer. J. Bot.* **34**, 196–204.

Barnett, H. L. & Lilly, V. G. (1947*a*). The relation of thiamin to the production of perithicia by *Ceratostomella fimbriata*. *Mycologia*, **39**, 699–708.

Barnett, H. L. & Lilly, V. G. (1950). Nutritional and environmental factors influencing asexual sporulation of *Choanephora cucurbitarum* in culture. *Phytopathology*, **40**, 80–89.

Barnett, H. L. & Lilly, V. G. (1955). The effects of humidity, temperature and carbon dioxide on sporulation of *Choanephora cucurbitarum*. *Mycologia*, **47**, 26–29.

Bishop, O. N. (1947). *Penicillium lanoso-grisellum* Biourge, a soil fungus antagonistic to *Phytophthora cryptogea*. B.Sc. Thesis, University of Bristol.

Brian, P. W., Curtis, P. J. & Hemming, H. G. (1946). A substance causing abnormal development of fungal hyphae, produced in *Penicillium Janczewskii* Zal. I. Biological assay, production and isolation of 'curling factor'. *Trans. Brit. mycol. Soc.* **29**, 173–187.

Brian, P. W., Curtis, P. J. & Hemming, H. G. (1949). A substance causing abnormal development of fungal hyphae, produced in *Penicillium Janczewskii* Zal. III. Identity of 'curling factors' with griseofulvin. *Trans. Brit. mycol. Soc.* **32**, 30–33.

Buller, A. H. R. (1909–34). *Researches on fungi*, I–VI. London.

Buston, H. W., Jabbar, A. & Etheridge, D. E. (1953). The influence of hexose phosphates, calcium and jute extract on the formation of perithecia by *Chaetomium globosum*. *J. gen. Microbiol.* **8**, 302–306.

Cantino, E. C. (1951). Metabolism and morphogenesis in a new *Blastocladiella*. *Leeuwenhoek ned. Tijdschr.* **17**, 59–96.

Cantino, E. C. (1952). The biochemical nature of morphogenetic pattern in *Blastocladiella*. *Amer. Naturalist*, **86**, 399–404.

Cantino, E. C. (1953). The role of metabolism and α-ketoglutarate oxidase in the growth and differentiation of the aquatic Phycomycete *Blastocladiella emersonii*. *Trans. N.Y. Acad. Sci.*, Ser. II, **15**, 159–163.

Cantino, E. C. (1954). The role of metabolism in morphological differentiation of the water fungus, *Blastocladiella emersonii*. *Rap. et Commun. VIII Congr. Int. Bot. Paris*, Sect. 19, 98–100.

Garrett, S. D. (1953). Rhizomorph behaviour in *Armillaria mellea* (Vahl) Quél. I. Factors controlling rhizomorph initiation by *A. mellea* in culture. *Ann. Bot., Lond.*, N.S., **17**, 63–79.

Goldring, D. (1936). The effect of environment on the production of sporangia and sporangiola in *Blakeslea trispora* Thaxter. *Ann. Mo. bot. Gdn*, **23**, 527–41.

Gregory, P. H. (1939). The life-history of *Ramularia vallisumbrosae* Cav. on *Narcissus*. *Trans. Brit. mycol. Soc.* **23**, 24–54.

Grove, S. F. & McGowan, S. C. (1947). The identity of griseofulvin and 'curling factor'. *Nature, Lond.*, **160**, 572.

Hamada, M. (1940). Physiologische-morphologische studien über *Armillaria mellea* (Vahl) Quél., mit besonderer Rücksicht auf die Oxalsäurebildung. *Jap. J. Bot.* **10**, 387–404.

Hawker, L. E. (1944). The effect of vitamin B_1 on the utilisation of glucose by *Melanospora destruens* Shear. *Ann. Bot., Lond.*, N.S., **8**, 79–90.

Hawker, L. E. (1947). Further experiments on growth and fruiting of *Melanospora destruens* Shear in the presence of various carbohydrates, with special reference to the effects of glucose and sucrose. *Ann. Bot., Lond.*, N.S., **9**, 245–259.

Hawker, L. E. (1950). *Physiology of fungi*. London.

Hirsch, H. M. (1954). Environmental factors influencing the differentiation of protoperithecia and their relation to tyrosinase and melanin formation in *Neurospora crassa*. *Physiol. Plant.* **7**, 72–97.

Hirsch & Westergaard, M. (1954). Environmental and genetic control of differentiation in *Neurospora*, *Colston Papers*, *Bristol*, **7**, 171–183.

Keyworth, W. G. (1942). The occurrence in artificial culture of tremelloid outgrowths on the pilei of *Coprinus ephemerus*. *Trans. Brit. mycol. Soc.* **25**, 307–310.

Klebs, G. (1898, 1899, 1900). Zur physiologie der Fortpflanzung einiger Pilze. *Jb. wiss. Bot.* **32**, 1–70; **33**, 513–593; **35**, 80–203.

Lilly, V. G. & Barnett, H. L. (1949). The influence of concentration of nutrients, thiamin, and biotin upon growth and formation of perithecia and ascospores by *Chaetomium convolutum*. *Mycologia*, **41**, 186–196.

Lilly, V. G. & Barnett, H. L. (1951). *Physiology of the fungi*. New York.

Nicholls, V. O. (1952). Studies on the association between certain soil fungi and the roots of some members of the Liliiflorae. Ph.D. Thesis, University of Bristol.

Pasteur, L. (1876). *Études sur la Bière*. Paris.

Perkins, S. J. (1952). The effect of low temperature on the formation of zygospores of some members of the Mucorales. B.Sc. Thesis, University of Bristol.

Plunkett, B. E. (1953). Nutritional and other aspects of fruit-body production in pure cultures of *Collybia velutipes* (Curt.) Fr. *Ann. Bot., Lond.*, N.S., **17**, 193–216.

Plunkett, B. E. (1954). The aeration complex of factors and fruit-body formation in pure cultures of Hymenomycetes. *Rap. et Commun. VIII Congr. Int. Bot. Paris*, Sect. 19, 101–102.

Raper, J. R. (1952). Chemical regulation of sexual processes in the Thallophytes. *Bot. Rev.* **18**, 447–545.

Raper, K. B. & Thom, C. (1949). *Manual of the Penicillia*. Baltimore.

Roberts, P. (1954). The effect of low temperature on zygospore production in *Mucor sexualis*. B.Sc. Thesis, University of Bristol.

Steinberg, R. A. (1919). A study of some factors in the chemical stimulation of growth of *Aspergillus niger*. *Amer. J. Bot.* **6**, 330–372.

Steinberg, R. A. (1939). Growth of fungi in synthetic nutrient solutions. *Bot. Rev.* **5**, 327–350.

Thom, C. & Raper, K. B. (1945). *Manual of the Aspergilli*. Baltimore.

Townsend, B. B. (1952). The morphology and physiology of the sclerotia and rhizomorphs of certain fungi. Ph.D. Thesis, University of Bristol.

Townsend, B. B. (1954). Morphology and development of fungal rhizomorphs. *Trans. Brit. mycol. Soc.* **37**, 222–233.

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MERIA LARICIS ON JAPANESE AND HYBRID LARCH IN BRITAIN

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The occurrence of *Meria laricis* Vuill. on Japanese larch (*Larix leptolepis*) and Hybrid larch (*L. Eurolepis*) in 1954 is recorded. These are believed to be the first British records on these hosts though the parasite has been recorded frequently causing Leaf Cast of European larch (*L. decidua*) in Britain.

Introduction

Meria laricis Vuill. causes discoloration and browning of the needles of European larch (*Larix decidua* Miller), the symptoms becoming first apparent about the beginning of May. The infection may occur on any part of the needle, the part above dying, so that the needles are browned from the tips downwards. On needles which remain attached to the young plants during the winter, isolated brown spots may be seen, because in cold weather the spread of the fungus is slower and the death of the needles less rapid.

The fructifications are borne usually on the undersurface of the needles, emerging from the stomata, and the spore clusters may be seen as rows of dark blue spots when stained with cotton blue or picro-aniline-blue. Before spore formation takes place several hyphae grow towards a stoma and form a coiled mass beneath it. Subsequently, the conidiophores emerge through the stomata as aseptate hyphae, later becoming one- to three-septate. Each cell bears a succession of spores on a sterigma near its apex. The spores are slightly constricted at the centre, and a septum is laid down at this point before germination. Vuillemin put the fungus into the family Hypostomaceae, regarding it as a simple Basidiomycete, closely related to the Ustilaginales. Hartig, Saccardo, Lindau and Fiori put it in the Fungi Imperfecti. The taxonomic status of the fungus is still obscure.

The fungus can be very troublesome on nursery stock, but is of no consequence on older trees. It is not usually the direct cause of death but increases the number of rejected plants. This is due to the weakening effect of the defoliation which it causes. The severity of attack is influenced by the weather. Damp weather favours the disease, while conversely a dry season checks it almost completely. Peace & Holmes (1933) carried out infection experiments at different temperatures. Infection was general between 10 and 25° C. (50–77° F.), slight at 5 and 0° C. (41 and 32° F.), and did not occur at 30° C. (86° F.); thus infection appears to occur under most non-winter temperatures.

Occurrences in 1954

In the summer and autumn of 1954, the specimens shown in Table 1 were examined. The Bareagle, Fleet, and Davidstow Japanese larch (*Larix leptolepis* (Sieb. & Zucc.) Murr.) all came from different sources into the nurseries. There seems no possibility, therefore, of a common source of the attack on Japanese larch.

Table 1

	Nursery	Species	Status of *Meria*	Cultured	Spore size (25 spores)
1	Davidstow, Cornwall	Japanese larch	Present	*Meria* not cultured	
2.	Fleet, Kirkcudbrightshire	Japanese larch, European larch	*Meria* present on both	Cultures obtained from both	On European larch. 6–10 × 2·5–3·0 μ On Japanese larch. 6–10 × 2·5–3·0 μ
3.	Bareagle, Wigtownshire	Japanese larch, Hybrid larch, European larch	*Meria* present on all three	Cultures obtained from both	From cultures of European larch 7–9 × 2·5–3·0 μ On Japanese larch 6–9 × 2–3 μ
4.	Glasfryn, Caernarvonshire	Hybrid larch, Japanese larch	On Hybrid, but not on Japanese larch	No cultures made	—
5.	Alice Holt, Hampshire	European larch	Present	Cultured	8–10 × 2·5–3·0 μ

It should be made clear that the word Hybrid larch (*Larix Eurolepis* Henry) here includes trees of the first, second or subsequent generation of the cross *Larix decidua* × *L. leptolepis*, and possibly back-crosses with either parent.

Peace & Holmes (1933) obtained two distinct strains of *Meria laricis* in culture. One strain, faster growing and profusely sporing, was termed the 'a' strain, and the other, slower growing and sparsely sporing, the 'b' strain. The 'a' strain produces a deliquescent growth on malt-agar, the surface of the culture being one mass of spores. It hardly colours the main mass of the medium until it is very old, when on drying out it turns it a dark brown. The top edge of the agar slant soon turns a light green, and this may in some cases extend a certain way down the medium. The 'b' strain has a characteristically dry appearance, with a slight fluffy surface mycelium. It rapidly changes the colour of the medium through dark greenish brown to almost black. The top end of the slant at the same time turns a brilliant emerald green. In isolations from nature the 'b' strain tends to occur rather more commonly than the 'a'. Cultures made from both European and Japanese larch in 1954 were all of the 'b' strain. No differences could be observed between the cultures obtained from the two larch species.

Distribution of the fungus

Peace & Holmes (1933) list records of the fungus in France, Germany, Austria, Italy and Norway. All these appear to refer to the disease on European larch. In this country it has been recorded on European larch and Western larch (*L. occidentalis* Nutt.), and once on a single needle of Hybrid larch.

Peace & Holmes carried out also a large number of inoculation experiments with different species of larch. Inoculations were successful on European larch and Western larch. Only one needle of Japanese larch was ever successfully infected, and very large numbers of inoculations on this species, made by methods which gave ample infection on European larch, were unsuccessful. *L. sibirica* Ledeb. and *L. gmelini* (Rupr.) Litvin could not be infected by these methods. In these cases the spores germinated, but failed to penetrate the needle.

Robak (1946) recorded *Meria laricis* on European, Siberian and Japanese larch in Norway. The damage to Japanese larch was unimportant. Langner (1952) says 'In 1951 a needle browning and necrosis of undetermined fungal origin severely attacked nursery material of European larch in Western Germany but not Japanese larch. Interspecific hybrids proved to be resistant when *L. leptolepis* was used as the female parent and susceptible when it served as the male, suggesting that the differential reactions to the disease are genetically conditioned.' Although in this case the fungus was not identified it was probably *Meria laricis*. In Czechoslovakia (Prichoda, 1954) the fungus was recorded on European larch 50 years ago and recently in 1952 or 1953. It occurs in the U.S.S.R. on Siberian Larch (Orloś, 1951). Recently a sample of infected larch was received from H. Orloś in Warsaw. This is the first record of *M. laricis* for Poland.

Discussion

It would appear that the above records are the first in Great Britain for *Meria laricis* on Japanese larch (*L. leptolepis*). It is the first occasion on which it has been recorded as doing appreciable damage to Hybrid larch, though as Langner (1952) indicates, the genetical constitution of the hybrid may have a major influence on its susceptibility or resistance.

The damage on the Japanese larch in all the nurseries was quite noticeable, but not generally serious. In one case it had more or less disappeared by the end of the summer.

Peace & Holmes (1933) carried out a number of experiments designed to elucidate the resistance to the fungus, which they observed in Japanese larch. No satisfactory explanation was found, but it was observed that a sterile filtrate of expressed, undiluted sap from needles of Japanese larch did delay the germination of *Meria* spores.

For the moment no explanation is advanced to account for the sudden appearance of the fungus on hosts which had hitherto been regarded as virtually immune. It is hoped that further observation in infected nurseries, combined with culturing and the cross-inoculation experiments, will throw more light on this interesting problem.

Acknowledgements are due to the Forestry Commission, under whose auspices the work was carried out.

REFERENCES

Langner, W. (1952). Reziprok unterschiedliches Verhalten von Lärchenbastarden gegen eine Nadelerkrankung. *Z. Forstgenet.* **1**, 78–81.

Orloś, H. (1951). *Przewodnik do oznaczania chorób drzew i zgnilizny drewna.* 190 pp. Warsaw.

Peace, T. R. & Holmes, C. H. (1933). *Meria laricis*, the leaf cast disease of Larch. *Oxford Forestry Memoirs*, no. 15, 28 pp.

Prichoda, A. (1954). Sypavka modřinu způsobená houbou *Meria laricis* Vuill. *Lesn. Prace*, **33**, 364–368.

Robak, H. (1946). Tre skogsykdommer som hittil har vaert lite kjent eller påaktet i Norge. *Tidsskr. Skogbruk.* pp. 323–334.

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ZYGOSPORES IN *PIPTOCEPHALIS CYLINDROSPORA* BAIN.

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(With Plate 1 and 1 Text-figure)

The features and development of the zygospores of *Piptocephalis cylindrospora* are described. The species is homothallic, and zygospores are formed by the delimitation of buds arising around the fusion points of heterogametangia. The synonymity of *P. cylindrospora* and *P. tieghemiana* and the observations of Matruchot on the zygospores of the latter are discussed.

A chance inspection of a potato-dextrose agar culture of *Piptocephalis cylindrospora* Bainier on *Circinella* (?) *tenella* (Ling-Young) Zycha revealed the presence of zygospores. Zygospores have only been reported in two species of *Piptocephalis*. Brefeld (1872) discovered those of *P. freseniana* De Bary & Wor., and Matruchot (1899, 1900) those of *P. tieghemiana* Mat. The latter species is considered by Zycha (1935) and Naumov (1939) as being identical with *P. cylindrospora* Bainier.

Description

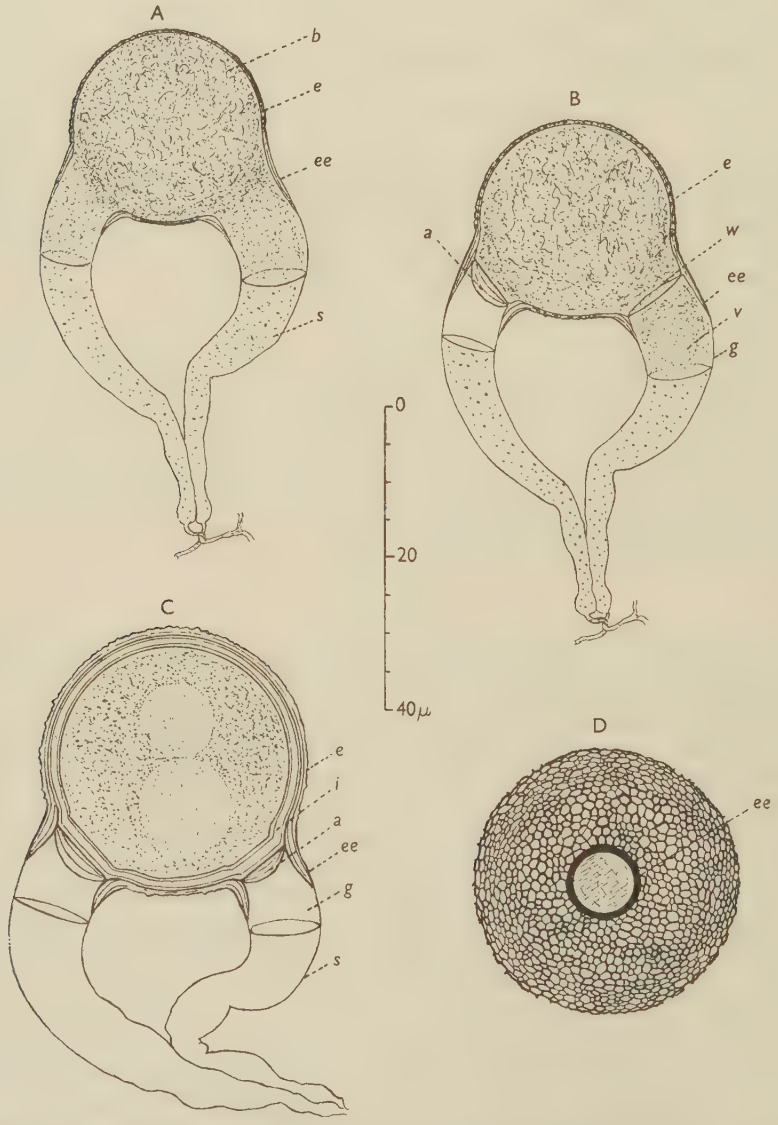
Mature, spherical zygospores are held between pairs of structures which are curved in both face and side views (Pl. 1, fig. 6; Text-fig. 1 C). Each such structure consists of a suspensor and a 'gametangial remnant'. The exospore, with two or three strata, appears to be completely penetrated by a reticulum of variously sized pits with regular, or irregular, pentagonal or hexagonal walls.

After zygospore delimitation, the exospore initially acquires an orange colour, then becomes more and more carbonaceous until finally it is almost black. The thick elastic endospore possesses four layers (Pl. 1, fig. 8; Text-fig. 1 C). The fine, outermost layer is carbonaceous at maturity and bears on its surface a very delicate replica of the exospore architecture. Of the other three layers, the outer and inner are each approximately half as thick as the stratified middle layer.

Areas of the zygospore wall occupied by gametangial remnants possess characteristic features described later. In surface view, such an area appears as an annulus (Text-fig. 1 D). Zygospores exhibit a size-range of (15) 27–37 (42 μ) and the larger have walls 2–3 μ thick. Zygospores usually contain large oily globules.

Development

Copulation branches appear to arise from separate mycelia, but, since single-spore cultures show the species to be homothallic, this observation requires confirmation. It is admitted that the coiling of mycelia and their



Text-figure 1. *Piptocephalis cylindrospora*. A, zygospore bud; B, delimitation of zygospore bud A; C, section of mature zygospore showing disposition of walls; D, surface view of mature zygospore showing reticulate architecture of the exospore and the annular appearance of the exospore extension. *a*, appositional thickening; *b*, zygospore bud; *e*, exospore; *ee*, exospore extension; *g*, gametangial remnant; *i*, endospore; *s*, suspensor; *v*, vacuole; *w*, separating wall.

distal enlargement into progametangia have not been followed. The youngest stages seen to date show swollen progametangia in contact. Differentiation of the latter into suspensors and gametangia shows that this species is heterogametangial (Pl. 1, fig. 1).

Budding around the point of fusion follows the breakdown of the contact walls and the bud soon becomes even and uniform, yet with greater development above than below the point of fusion. Thus, signs of the greater contribution to the bud by the larger gametangium are soon lost. (Pl. 1, fig. 2, 3). Nipples, often seen above and/or below the point of fusion prior to budding, have no developmental significance but merely represent disparities in gametangial size.

As in *P. freseniana*, the exospore makes an early architectured appearance during budding. It is remarkable that this architecture is confined to the zygospore bud and does not occur on exospore extensions into what will be gametangial remnants. Where the zygospore bud becomes confluent with future gametangial remnants, the exospore exhibits a heavier circumferential disposition. Such perimeters mark the positions of future cleavage planes in which walls are deposited delimiting the bud towards the end of its expansion. The deposition of such walls has only once been found to be simultaneous (Pl. 1, fig. 5; Text-fig. 1 B). The residual protoplasm in the gametangial remnants becomes vacuolate and condenses to give mounds of appositional thickening on the separating walls. The amount of appositional thickening varies not only between zygospores but also between the gametangial remnants of a single zygospore. But it is always present and often adopts a characteristic form (Pl. 1, fig. 5; Text-fig. 1 C). The endospore appears last, but the deposition of its separate layers has not been satisfactorily followed (Pl. 1, fig. 8).

Where the zygospore is delimited from a gametangial remnant the complete wall is compounded of endospore, a separating layer, a mound of appositional thickening and a tapering cylindrical flank of exospore extension. Such a flank, in surface view, appears as an annulus (Text-fig. 1 D). The protoplasm in the suspensors breaks down before or after zygospore delimitation and contributes to the formation of one or two additional septa and/or basal hypertrophy.

A mature zygospore still reflects the heterogametangial condition by the differences in dimensions of its gametangial remnants.

Azygospores are not common. They can be formed in one or other or both gametangia, but the usual rule is azygospore formation in the larger gametangium. Where both gametangia form azygospores, the heterogametangial condition is well emphasized. Azygospores possess only one annulus (Pl. 1, fig. 7).

Discussion

Matruchot (1899, 1900), in his description of the zygospores of *P. tieghemiana*, stated that the exospore architecture consists of numerous small rounded tubercles; that the gametangia are equal and similar; that the zygospore is formed between copulation branches; and, therefore, that zygospore formation differs fundamentally from that in *P. freseniana*, where the zygospore is derived from an apical bud at the point of fusion.

The foregoing brings into question either the synonymity of *P. cylindrospora* and *P. tieghemiana* or the completeness of Matruchot's observations. Accepting the synonymity of the former, it is easily appreciated why Matruchot described the exospore as tuberculated. In profile it appears so, but surface and section examinations show that it is pitted. Yet, if he could pass an opinion on this fine architecture, how could he have failed to mention or figure the characteristic form of the walls delimiting the zygospore? And how could he have overlooked the heterogametangial condition and the true method of zygospore formation?

P. cylindrospora and *P. freseniana* show no fundamental differences in their modes of zygospore formation. The latter has a zygospore bud above the point of gametangial fusion, whereas the former has a bud around the point of fusion yet with greater development above than below it. An unusual zygospore is featured exhibiting marked apical budding (Pl. 1, fig. 8). Zygospore formation in *P. cylindrospora* is best considered as intermediate between that occurring in *Phycomyces*, *Blakeslea*, etc., where the zygospores arise from complete fusion cells, and that in *P. freseniana*, *Syncephalis*, etc., where they arise as distinct buds from fusion cells.

Whilst one must agree with Blakeslee (1904) that Matruchot's account is not detailed, is it likely that such a disparity should exist between two accounts of the same zygospore? The acceptance of Matruchot's account naturally disputes the synonymity of *P. cylindrospora* and *P. tieghemiana*.

REFERENCES

Brefeld, O. (1872). *Mucor mucedo, Chaetocladium Jonesii, Piptocephalis freseniana, Zygomyceten. Bot. Unters. über Schimmelpilze*, **1**, 1–64.

Blakeslee, A. F. (1904). Sexual reproduction in the Mucorineae. *Proc. Amer. Acad. Arts Sci.* **40**, 205–319.

Matruchot, L. (1899). Sur un nouveau mode de formation de l'œuf chez les Piptocephalis. *C.R. Acad. Sci., Paris*, **129**, no. 24.

Matruchot, L. (1900). Notes mycologiques: *Piptocephalis tieghemiana*. *Bull. Soc. mycol. Fr.* **16**, 58–64.

Naumov, N. A. (1939). *Clés des Mucorinées*. 137 pp. Paris: P. Lechevalier.

Zycha, H. (1935). Mucorineae. *Kryptogamenflora der Mark Brandenburg*, **6***a*, 264 pp.

EXPLANATION OF PLATE 1

Piptocephalis cylindrospora

Fig. 1. Heterogametangia in contact.

Fig. 2. The commencement of budding after fusion. The slight constriction on the right indicates the point of fusion.

Fig. 3. A bud with architectured exposure and non-architectured extensions into future gametangial remnants. The differences in dimensions of the latter are reminders of the heterogametangial condition. The two hyphae on the left represent the separate mycelia from which the copulation branches arose.

Fig. 4. A later stage of budding focused to show on the right an exospore extensions into a future gametangial remnant.

Fig. 5. Delimitation of zygospore bud. A separating wall is faintly indicated on the right; whilst a separating wall and appositional thickening are present on the left.

Fig. 6. A mature zygospore. Note the basal hypertrophy in the left suspensor, the faintly indicated reticulum of pentagonal or hexagonal pits on the exospore surface, the exospore extensions and associated appositional thickening in the gametangial remnants.

Fig. 7. Azygospore formation in the larger gametangium.

Fig. 8. An unusual zygospore showing marked apical budding. The endospore is well shown on the bottom left.

(Fig. 1, 4–6, × 875; figs. 2, 3, 7, × 950; fig. 8, × 1000.)

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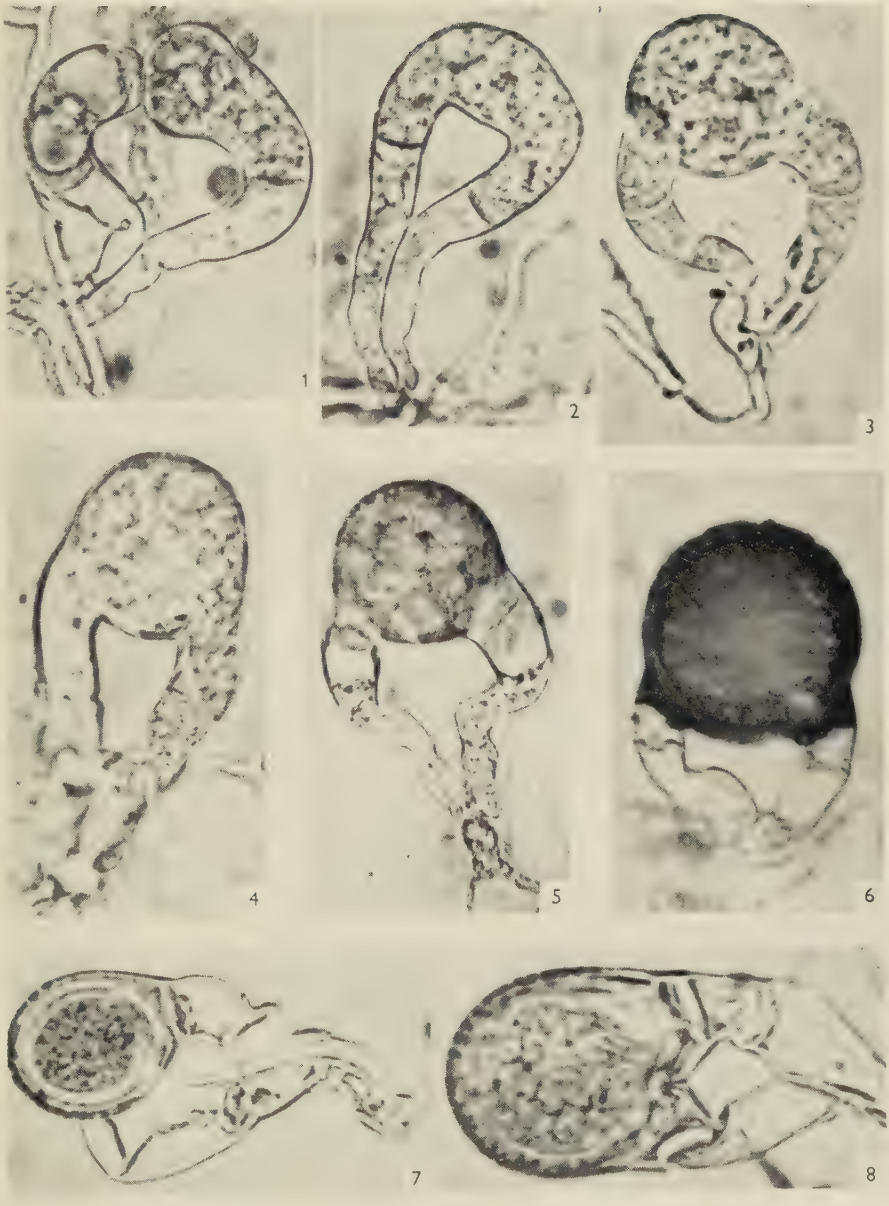

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SOME PYCNIDIAL FUNGI ON *CAREX*

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(With 10 Text-figures)

The following species of pycnidial fungi with colourless spores occurring on dead leaves of *Carex* spp. in Britain are described and illustrated: *Diplodina teretiuscula* (Sacc. & Roum.) Died., *Ascochyta sodalis* Grove, *Stagonospora caricinella* Brun., *S. vitensis* Unam., *S. caricis* (Oud.) Sacc., *S. paludosa* (Sacc. & Speg.) Sacc., *S. gigaspora* (Niessl) Sacc. and *S. anglica* sp.nov.

Cultural features and fruiting behaviour of single spore isolates of these fungi (except *S. gigaspora*) on agar media and on sterilized *Carex riparia* leaves are described. The formation of perfect stages in culture by *Ascochyta sodalis* and *Stagonospora caricinella* is noted.

Introduction

Many species of Sphaeropsidales are to be found in the large fungus flora of dead or moribund leaves and stems of reedswamp, marsh and fen plants. Most of these are Monocotyledons such as *Typha* spp., *Phragmites communis* and members of the Juncaceae and Cyperaceae.

This paper is confined to fungi on certain species of *Carex*. This genus is the largest in the British flora with about 76 species and numerous hybrids (Clapham, Tutin & Warburg, 1952). They are present in many types of habitat but most occur at the edges of lakes and streams or in other places such as low-lying hollows on heathland, and dune slacks where surface water is present, at least during winter months. All the fungi to be described have been found in a healthy condition on dead submerged or saturated leaves of sedges at various times.

Except where indicated, all collections have been made in south-west Middlesex and the adjacent border areas of Buckinghamshire and Surrey during 1953 and 1954.

Methods

Isolations were made by spreading a spore suspension in sterile water over a film of 2 % malt agar on a glass slide. After 24 hr. at room temperature single, germinating spores were removed under low power of the microscope with a stout flat-ground needle. Stock cultures were kept on 2 % malt agar slopes at about 12° C. Mycelial transfers were made every 3–4 months.

Fruiting was generally poor on the media used (2 % malt agar, oatmeal agar, potato-dextrose agar) and tended to decline with age of the isolate. Most of these cultures, sterile on the agar media, produced pycnidia when grown on pieces of sterilized leaves of *Carex riparia* in the following manner (Fig. 1). A 1 × 1 in. aluminium cap was inverted and filled with oatmeal

agar and placed in a tall 400 ml. beaker fitted with a Petri dish lid. This was autoclaved, and three pieces of sterilized *C. riparia* leaf, each about 10 cm. long, were stuck vertically into the agar. Fresh, unblemished leaves were used and sterilized by autoclaving in boiling tubes half filled with water. The agar was inoculated and a small quantity of sterile water was introduced into the bottom of the beaker. After the mycelium had grown some distance up the leaves the water-level was raised above the rim of the cap to prevent further drying.

A standard method of spore measurement was adopted. Where possible living spores were used. Ten pycnidia were broken open in a drop of water on a slide and the contents thoroughly stirred up before a cover-slip was applied. 100 spores taken at random were then drawn and measured using a camera lucida. As the sample number was large the standard deviation was calculated from the formula

$$Sx = \sqrt{\left[\frac{\Sigma f(x-x_a)^2}{n} - \left(\frac{\Sigma f(x-x_a)}{n}\right)^2\right]}$$

without applying Bessel's correction.

In the seven species studied in culture, colony descriptions are based on at least four separate single-spore isolates from at least two collections made at different times (except in *Stagonospora caricis* where the four single spore isolates were from one collection). Parallel cultures were grown at 20–23° C. in light of approximately 100 f.c. on plates poured from a single batch of agar medium.

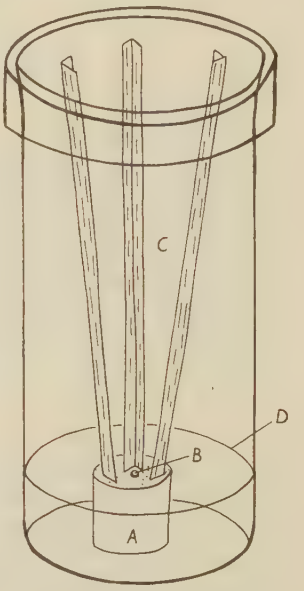

Fig. 1. Apparatus used for growing fungi on sterile pieces of *Carex riparia* leaf. *A*, aluminium cap containing oatmeal agar; *B*, inoculum; *C*, pieces of autoclaved *Carex riparia* leaf; *D*, level to which water is added.

However, for greater accuracy, growth rates were compared on 2 % malt agar in the dark in a 20° C. incubator housed in a cold-room running at 12–15° C. Three colonies of each of two single spore isolates were used for each species. Diameters were measured daily along two lines at right angles to each other. For both description and growth rate on agar, colonies were grown from standard 4 mm. diameter inocula.

Diplodina teretiuscula (Sacc. & Roum.) Died.

This species has been found several times, particularly from November to February, on the dead lower leaves and leaf sheaths of *Carex hirta* and *C. disticha*. These two species often grow in damp hollows, and during the winter the dead remains of the previous season's growth form a thick litter which is always saturated and may be submerged in surface water for considerable periods. It is on these saturated dead parts that the fungus has been found in a healthy condition. A collection has also been made

from the dead leaf-sheaths of *Carex* sp. from Kenmare, Co. Kerry, Ireland in mid-August.

The pycnidia are densely scattered on both sides of the dead leaves and on the exposed parts of the leaf-sheaths (Fig. 2 A, B). They are non-erumpent, spherical, 150–260 μ in diameter (average of 50, 196 μ) with ostioles up to 30 μ across (Fig. 2 D). The wall is 35–45 μ thick and consists of several layers of flattened cells. The cells of the outer wall layer are angular, isodiametric 3–12 μ across (Fig. 2 C). When crushed and viewed by transmitted light under low power of the microscope the wall is Mummy Brown in colour.*

The spores (Fig. 2 E) are straight, cylindrical with rounded ends and each has a single median septum at which the thin smooth wall is not constricted. The spores are hyaline with two or three small oil drops per cell. Dimensions of 100 random spores from two different collections are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Carex hirta	10·5–14·0	11·9	0·77	2·0–3·5	2·7
C. disticha	10·0–13·0	11·2	0·63	2·0–3·5	2·8

Of the many species of fungi on Monocotyledons assigned to the genera *Ascochyta* and *Diplodina* only a few have been recorded on the Cyperaceae. The spores of *A. socialis* Sacc. (15–16 × 5 μ) (Saccardo, 1884) and *A. caricis* Lamb. & Fautr. (12–14 × 3–4 μ) (Saccardo, 1899) are too wide to fit this material, and those of *A. lacustris* Passer. (5–8 × 2·5–3 μ) (Saccardo, 1892) are too short. *Diplodina caricis* Grove is described on *Carex arenaria* from Buteshire with spores 13–16 × 3–4 μ (Grove, 1935). Syntype material of this has been examined at the Kew Herbarium. The measurements are in agreement with those published but in all respects this specimen appears identical with the syntype material of *Ascochyta sodalis* Grove described below.

Of other species on Monocotyledons in Britain *Diplodina teretiuscula* (Sacc. & Roum.) Died. on *Luzula* spp. is recorded as having spores 10–14 × 2·5–3 μ. Material of this on *Luzula pilosa* (J. W. Ellis, Worcester, 25 May 1912) at Kew herbarium has been examined. The spores (Fig. 2 F) appear identical with those of the fungus on *Carex*. They are cylindrical with rounded ends and mostly 1-septate, though rarely a spore with 2 septa is found. Dimensions of fifty random spores in lactophenol are: length, 10·0–14·0 (mean 12·8) μ; width, 2·0–3·5 (mean, 2·7) μ; septa, 1 (2).

Though living material on *Luzula* has not so far been examined, it appears logical at present to use this name for the fungus on *Carex*.

The spores germinate in water at room temperature within 24 hr. (Fig. 2 J). On 2 % malt agar a slow-growing circular colony with an irregular margin is formed. The aerial mycelium which is present to the edge of the colony is dense, cottony, Dusky Green-Grey to Hathi Grey in colour, with white patches. The reverse is black where there are grey regions of the aerial mycelium; the rest is white.

* All colour designations are according to Ridgway (1912).

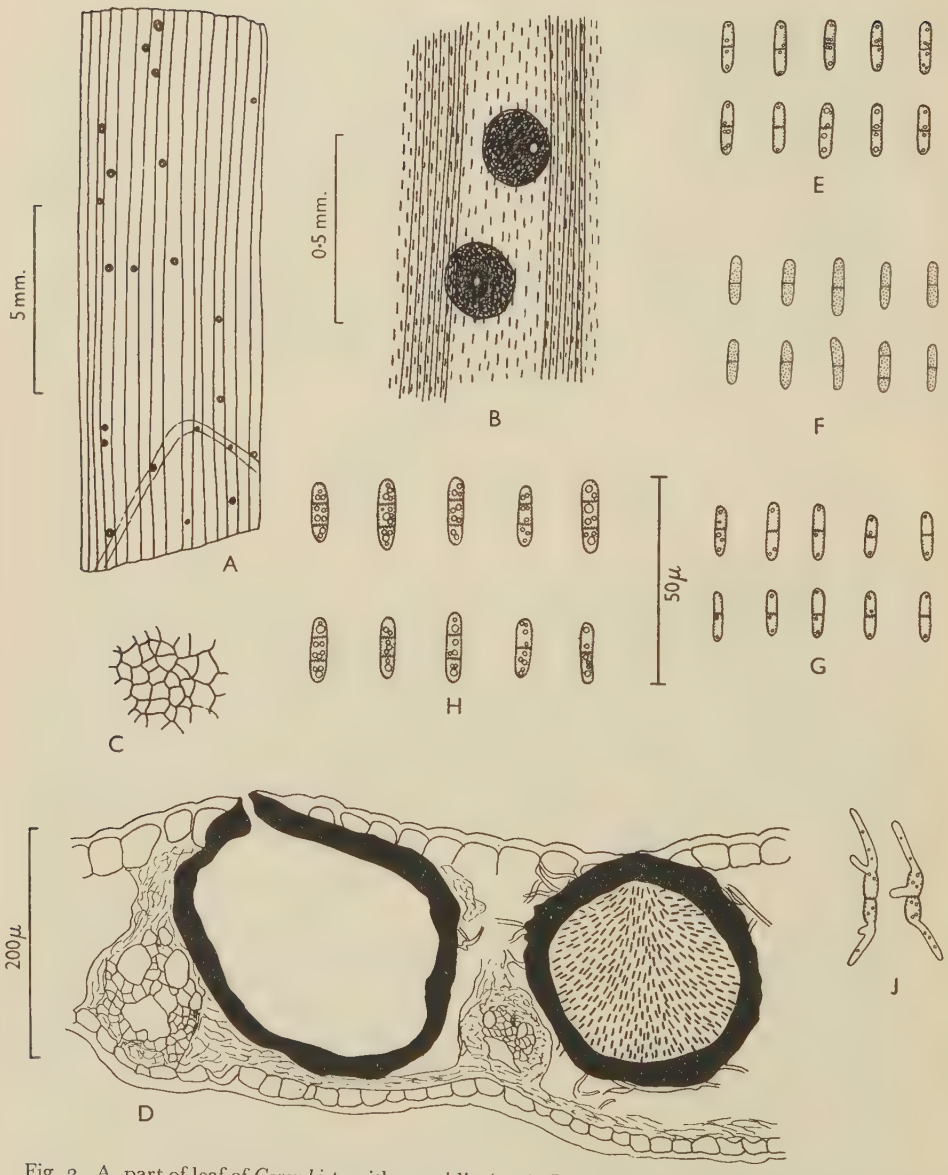

Fig. 2. A, part of leaf of *Carex hirta* with pycnidia (× 5); B, part of same (× 50); C, part of outer wall of pycnidium (× 555); D, vertical section of nearly mature and empty pycnidia (× 150); E, ten random living spores from nature (× 555); F, ten random spores of *Diplodina teretiuscula* (Sacc. & Roum.) Died. from specimen on *Carex riparia* in Kew Herbarium (× 555); G, ten random living spores from pycnidia formed on 2 % malt agar plate (× 555); H, ten random living spores from pycnidia formed on re-infected *Carex riparia* leaves (× 555); J, germinating spores after 24 hr. in water at room temperature (× 555).

Growth is similar on potato-dextrose agar but the aerial mycelium is not so thick. On oatmeal agar growth is more rapid and a colony is formed with a uniform colourless margin about 3 mm. wide devoid of aerial mycelium. The aerial mycelium is Dusky Green-Grey centrally grading to white at the margin. The reverse is black centrally, the rest white.

Old colonies on the three media have a uniformly felted aerial mycelium Light Olive-Grey to Iron Grey colour in patches. The reverse is black. Fruiting on the agar medium used is poor but the spores present in the few pycnidia produced are similar to those in nature (Fig. 2 G).

On sterilized *Carex* leaves a thick, felted, Dark Olive-Grey mycelium is formed. Numerous pycnidia are produced by new isolates, though after 9 months in stock culture, subcultures form only a few. The spores (Fig. 2 H) are decidedly larger than those in nature and most are 2-septate though some typically small, 1-septate ones are found. This phenomenon is shown by both the English and Irish isolates. Dimensions of 100 spores taken at random from two different cultures is as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Oatmeal agar	10·0–14·0	11·9	0·5	2·0–3·5	2·7
Re-infected *Carex*	15·0–19·5	17·5	1·0	2·0–4·5	3·7

Material of this species has been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 59581), Kew Herbarium and the British Museum (Natural History).

Ascochyta sodalis Grove

This fungus has been found a few times in December and January on saturated dead leaves of *Carex ovalis* and *C. nigra*. The pycnidia (Fig. 3 A, B) are amphigenous and scattered more or less uniformly along the length of the dead leaf. Though small they are sufficiently numerous to give the leaf a speckled appearance, especially when wet. They are spherical, 65–120 μ in diameter (average of 50, 88 μ) with ostioles 10–15 μ across level with or slightly raised above the leaf surface (Fig. 3 C, D). The wall consists of several layers of flattened cells. The cells of the outer layer are characteristically large (10–15 μ across), angular and many contain conspicuous oil drops (Fig. 3 G). By transmitted light under low power of the microscope they are Raw Umber in colour.

The spores (Fig. 3 F) are ellipsoidal, with more or less pointed ends, straight or slightly curved and have a single, usually median septum. Living spores are slightly constricted at the septum, hyaline, and each cell contains two to six small refractive oil drops. Dimensions of 100 spores taken at random from two different collections are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Carex nigra	10·0–14·5	12·3	0·80	3·0–5·0	4·0
C. nigra	10·0–14·5	12·6	1·02	3·0–4·5	3·9

The only *Ascochyta* recorded on *Carex* in Britain is *A. sodalis* Grove. The description above agrees well with the original one (Grove, 1935), especially with regard to the small pycnidia with larger outer wall cells and the spore size which is given as 12–15 × 3–4·5 μ. The type material at Kew has been examined. This is on *C. arenaria* collected in July. The pycnidia are small and have large wall cells (Fig. 3 J). The spores (Fig. 3 H) are very

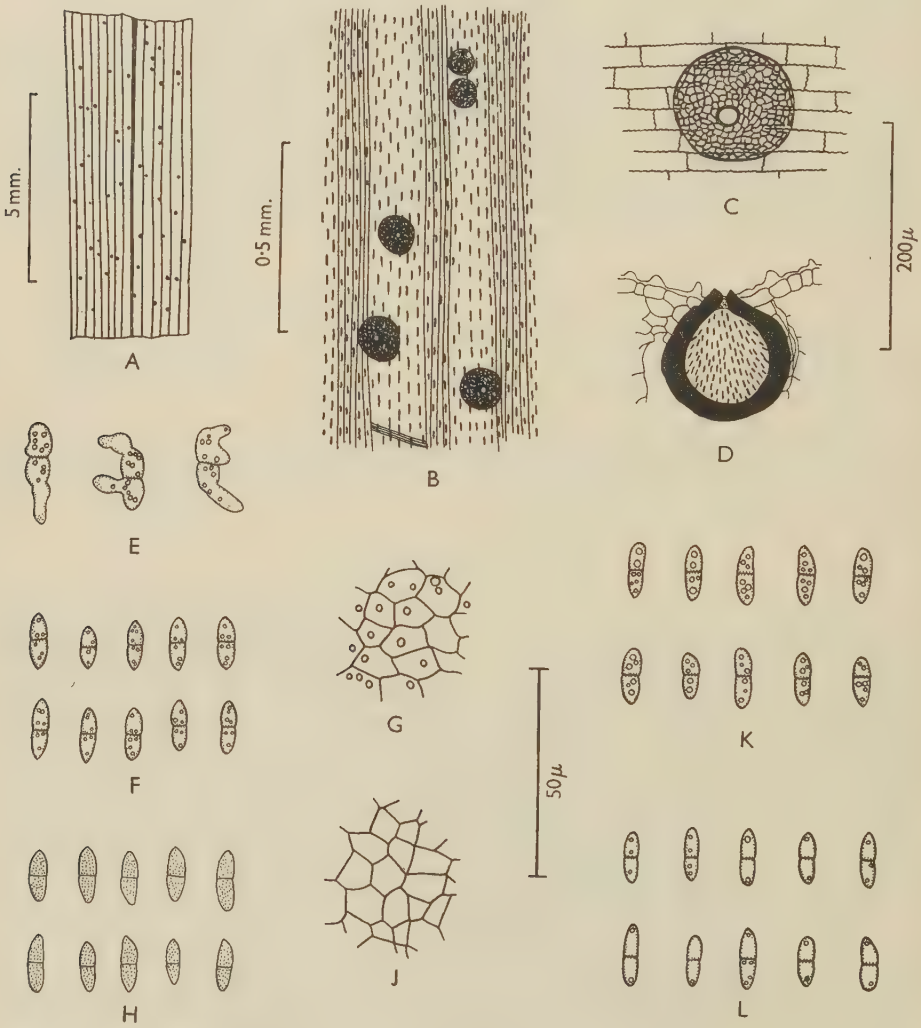

Fig. 3. A, part of leaf of *Carex nigra* with pycnidia (× 5); B, part of same (× 50); C, surface view of single pycnidium (× 150); D, vertical section of pycnidium (× 150); E, germinating spores after 24 hr. in water at room temperature (× 555); F, ten random living spores from nature (× 555); G, part of outer wall of pycnidium (× 555); H, ten random spores from type material of *Ascochyta sodalis* Grove mounted in lactophenol and cotton blue (× 555); J, part of outer wall of pycnidium from type material (× 555); K, ten random living spores from pycnidia produced on re-infected *Carex riparia* leaves (× 555); L, ten random living spores from pycnidia produced on 2% malt agar plate at 20° C. in light (× 555).

similar to those on *C. nigra* and mounted in lacto-phenol measure 11–15 × 3–5 μ (average of 50, 13·4 × 4·1 μ).

The spores germinate within 24 hr. in water at room temperature, forming one or two germ tubes per cell (Fig. 7 E). On malt agar growth is slow and results in an irregular colony with aerial mycelium present even at the ragged leading edge. The aerial mycelium is dense, cottony with patchy colouring of Castor Grey to Dawn Grey. The reverse is black except for a 2 mm. wide marginal zone which is white.

On potato-dextrose agar and oatmeal agar growth is more rapid giving a uniform, non-staling, round colony with a ragged edge. The aerial mycelium is uniform, densely cottony, white or with large Castor Grey areas. The reverse is black centrally with a 7–10 mm. wide marginal zone.

Old colonies tend to be overgrown with white patches. Fruiting is poor, though a few pycnidia may be formed on oatmeal or malt agar after 3 weeks.

On sterile *Carex* leaves a dense fluffy growth is formed. Beneath the leaf surface are numerous pycnidia with yellowish walls and containing typical spores.

Spores produced in culture (Fig. 3 K, L) are very similar, though slightly larger, than those from nature. Dimensions of 100 spores taken at random from two different cultures are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
2% malt agar	11·0–16·5	13·7	0·76	3·0–4·5	3·8
Re-infected *Carex*	10·5–17·0	13·0	0·95	3·0–5·0	3·8

A few perithecia have been found on 3-week-old colonies grown on oatmeal agar. The ascospores, which measure 17–22 × 5–7 μ (average of 25, 19·9 × 5·8 μ), are colourless, shortly fusiform, slightly curved, 1- to 3-septate and markedly asymmetrical. This perfect stage has not yet been identified, but appears to be a species of *Mycosphaerella*.

Pycnidial material of this fungus has been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 59584), Kew Herbarium and the British Museum (Natural History).

Stagonospora caricinella Brun.

This fungus has been found repeatedly on several of the waterside species of *Carex*. It occurs on the dead, *in situ* leaves at and below water-level, and also on dead drifting pieces of leaves and stems. It is particularly common on *C. acutiformis*, but it has also been found on *C. riparia* and *C. pseudocyperus*. On one occasion it was found on the dead leaves of a single plant of *Sparganium ramosum* which was growing in a closed community of *C. riparia* on which the fungus also occurred.

On dead *Carex* leaves the pycnidia (Fig. 4 A, B) are amphigenous though tending to be more abundant on the abaxial side on the lower parts. They are loosely gregarious but not confined to definite spots. The pycnidia are completely immersed, spherical with a diameter of 90–210 μ

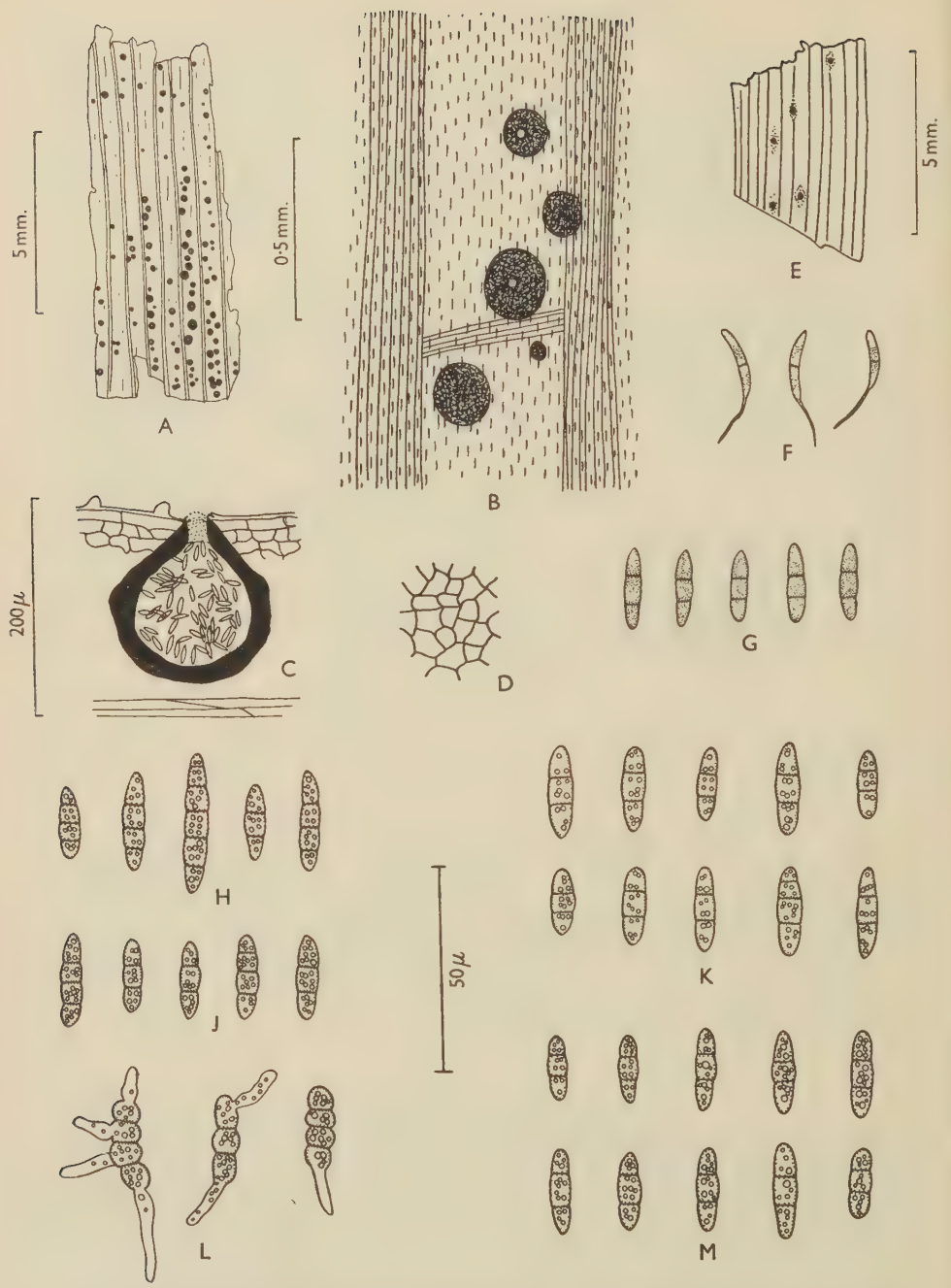

Fig. 4. A, part of leaf of *Carex acutiformis* with pycnidia (× 5); B, part of same (× 50); C, vertical section of pycnidium on leaf of *Carex acutiformis* (× 150); D, portion of outer wall of pycnidium (× 555); E, part of leaf of *Carex* sp. of type material of *Hendersonia macropus* B. & Br. from British Museum (Natural History) (× 5); F, spores from same mounted in lactophenol (× 555); G, spores of *Stagonospora caricinella* Brun. ex herb. Sydow from British Museum (Nat. Hist.) (× 555); H, five random spores from pycnidia of colony grown on oatmeal agar plate in light (× 555); J, five random spores from pycnidia on re-infected *Carex riparia* leaf (× 555); K, ten random living spores from nature (× 555); L, spores germinating after 18 hr. in water at room temperature (× 555); M, ten random spores from nature from pycnidia on *Sparganium ramosum* (× 555).

(average of 50, 154 μ) and open at the surface of the leaf by a small pore 15–20 μ in diameter (Fig. 4 C). The pycnidial wall is about 13 μ thick and consists of a few layers of flattened cells. The cells of the outer layer are polygonal, 5–10 μ across and have dark brown walls (Fig. 4 D). The crushed pycnidial wall has a Mummy Brown colour when seen by transmitted light under low power of the microscope. On *Sparganium* the pycnidia are similar but tend to be somewhat erumpent.

The spores (Fig. 4 K, M) are fusiform, straight with acute ends. They are colourless with thin smooth walls. The majority have two thin septa though occasionally a 3- or 4-septate spore is found. The spores are slightly constricted at the septa, a feature accentuated after a short time on mounting in water. Healthy mature spores contain four to ten obvious, refractive oil drops per cell.

Dimensions of 100 spores taken at random from three collections are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Carex pseudocyperus	18·0–26·0	21·0	1·75	4·0–6·0	5·2
C. riparia	17·0–27·0	19·7	1·95	4·0–6·0	4·8
Sparganium ramosum	14·0–22·0	17·6	1·66	4·0–5·0	4·6

Though the spores from the *Sparganium* material are smaller, cultural features leave no doubt that the same species is involved.

Of the four species of *Stagonospora* previously recorded on sedges in Britain (Grove, 1935), only *S. macropus*, originally described as *Hendersonia macropus* B. & Br. seemed a possible name for this fungus. However, the whole nomenclatural position of this species is very confused, largely because later workers appear neither to have read the original description carefully nor to have examined the type material. The first description of *H. macropus* (Berkeley & Broome, 1850) undoubtedly applies to the deposited syntype material, which I have examined, in the British Museum (Natural History) (Fig. 4 E). The spores (Fig. 4 F) are small (15–18 × 2–2·5 μ), curved and have a long attenuated pedicel (18 × 1 μ). It is clear that this species should not be in either *Hendersonia* or *Stagonospora*.

Fuckel (1869) further complicated matters by listing from Germany a fungus under the name *Darluca typhoidearum* (Desm.) B. & Br. f. *caricis* Fuckel which he considered, quite wrongly, synonymous with *H. macropus*. *Darluca typhoidearum* (Desm.) B. & Br. (= *Stagonospora typhoidearum* (Desm.) Sacc.) is morphologically very similar to the fungus being considered in this section and it may be that Fuckel's fungus on *Carex* was the same as mine.

Cooke (1871) transferred Berkeley's species to *Darluca*, but a specimen of *D. macropus* (B. & Br.) Cooke in the British Museum (Natural History), from Plowright's herbarium, does not coincide with either the type material or the present fungus.

Under *Stagonospora macropus* (B. & Br.) Sacc., Saccardo (1884), followed by later authors (Allescher, 1901; Migula, 1921), merely reproduced Berkeley's description unaltered. However, under the same name, Grove (1935) gave spore measurements (20–22 × 4–5 μ) and also indicated in

a footnote that he considered this fungus to be allied to *S. typhoidearum*. As with Fuckel's record, this suggests that Grove's fungus may have been the same as mine. Unfortunately, no herbarium material named as *S. macropus* by Grove has been available for examination.

Of the small-spored species of *Stagonospora* recorded on *Carex*, *S. strictae* (20–30 × 8–10 μ) (Ellis & Everhart, 1893) and *S. jaapii* Died. (28 × 7–7·5 μ) (Diedicke, 1915) fall in the same length range but have a greater width than the present species.

Brunaud gave a brief description, without figures, of *Stagonospora caricinella* Brun. found on dead leaves of *Carex riparia* in France (Brunaud, 1893). He described the spores as long, round at the extremities, hyaline, with five guttules, 20–22 × 3·5–4 μ. It has been impossible to obtain type material of this fungus, but a specimen under this name on *Carex acuta* (A. Ludwig, Lothringen, 8 April 1914) in the British Museum (Natural History) has been examined. The pycnidia are small, about 90 μ in diameter and the spores (Fig. 4 G) are hyaline, fusiform and 2-septate and though slightly smaller (15–19 × 4·5–6 μ) are very similar to those of the fungus under discussion. Thus, in spite of the absence of more reliable descriptions and material, it appears best to take up the name *S. caricinella* Brun. for this fungus rather than introduce a new one.

The spores germinate rapidly in water at room temperature, usually forming a single germ tube from each cell (Fig. 4 L). On 2 % malt agar a circular, non-staling colony is formed with an evenly ragged edge. The aerial mycelium is always sparse, cottony and is practically absent in old isolates except round the inoculum. It is Storm Grey centrally grading to white within 4 mm. of the leading edge. The reverse is black centrally grading to Deep Olive-Grey, with a white margin 9–10 mm. wide.

On potato-dextrose agar growth is more rapid, the aerial mycelium is denser and more uniform with the white and Storm Grey regions as concentric zones or clearly defined patches. The marginal 10 mm. is colourless without aerial mycelium. The black central region in reverse is less extensive than on malt agar.

On oatmeal agar the growth rate is the same as on malt agar but the colony has a more uniform .edge. The aerial mycelium is uniformly Storm Grey to Dawn Grey.

Recently isolated single spore cultures form numerous pycnidia on oatmeal agar and potato-dextrose agar but stock cultures maintained by mycelial transfers rapidly become sterile. After about 6 months very few pycnidia are produced on oatmeal agar, though fruiting is still good on re-infected sterile *Carex riparia* leaves. Cultures maintained for 18 months have become sterile even when transferred to *Carex* leaves.

In culture spore size is variable and the averages are higher than in nature. Dimensions of 100 spores taken at random from two cultures are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
2 % malt agar	19·0–32·0	24·6	3·2	4·0–7·0	5·0
Re-infected *Carex*	18·0–27·0	21·2	1·9	4·0–6·5	5·2
Re-infected *Carex*	23·0–43·0	30·0	4·1	5·0–8·0	6·1

Fresh isolates grown on *Carex* leaves produce a thick white to Storm Grey surface mycelium and abundant, typical pycnidia. Intermingled with these, and indistinguishable from them externally, are many perithecia. This perfect stage has been found on numerous occasions from six single-spore isolates from different sources, including two from *Sparganium*. In all these the perithecia are the same. The ascospores, which measure 24–32 × 6·5–9 μ (average of 100, 27·5 × 7·5 μ), are colourless, 1–3-septate, straight or slightly curved and slightly asymmetrical. This perfect stage has not yet been identified but appears to be a species of *Leptosphaeria*.

Specimens from collections in nature have been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 58658), Kew Herbarium and the British Museum (Natural History).

Stagonospora vitensis Unam.

This species has been found on a number of occasions on the dead lower leaves and leaf-sheaths of *Carex disticha*, *C. ovalis* and *C. hirta*. On the first of these it is often found associated with *Diplodina teretiuscula* (Sacc. & Roum.) Died. It is particularly abundant from November to February.

S. vitensis has also been found during January 1953 and 1954 on the dead basal portions of leaves of *Carex riparia* as much as 30 cm. below normal water-level.

The pycnidia are amphigenous and scattered (Fig. 5 A), and mostly spherical with a diameter of 150–350 μ (average of 50, 245 μ). On *C. hirta* and *C. disticha*, which have relatively narrow leaves, the larger pycnidia tend to be elongated due to the narrow available space in which they lie between two veins of the leaf (Fig. 5 B). The ostioles are flush with the leaf surface and have a diameter of 30–40 μ. Each ostiole region is firmly attached to the outermost cell layers of the leaf, the rest of the pycnidium projecting into the large air space below (Fig. 5 C). The wall of the pycnidium is about 10 μ thick and consists of several layers of thin plate-like cells, the outer layer being composed of irregular, polyhedral cells 8–14 μ across (Fig. 5 D). Under the low power of the microscope with transmitted light the wall of the crushed pycnidium is Tawny in colour.

The spores (Fig. 5 E) are hyaline with thin, smooth walls. They are cylindrical, mostly straight, rarely curved and have rounded ends. Most are 2- or 3-septate (very rarely 4-septate) and have several small refractive oil drops in each cell. When enclosed in the pycnidia the spores are embedded in mucilage and have straight parallel sides. This feature is seen in freshly discharged spores and is retained in lactophenol mounts. In water, however, living spores soon swell, their lateral walls bulge out and they appear very constricted at the septa.

Dimensions of 100 spores taken at random from three collections are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Width
Carex riparia base of leaf blade	20·0–30·0	24·0	2·22	4·0–5·0	4·5
C. hirta leaf sheath	18·0–28·0	23·9	1·83	4·0–6·0	5·2
C. ovalis leaf sheath	19·0–32·0	26·4	2·93	4·0–6·0	5·2

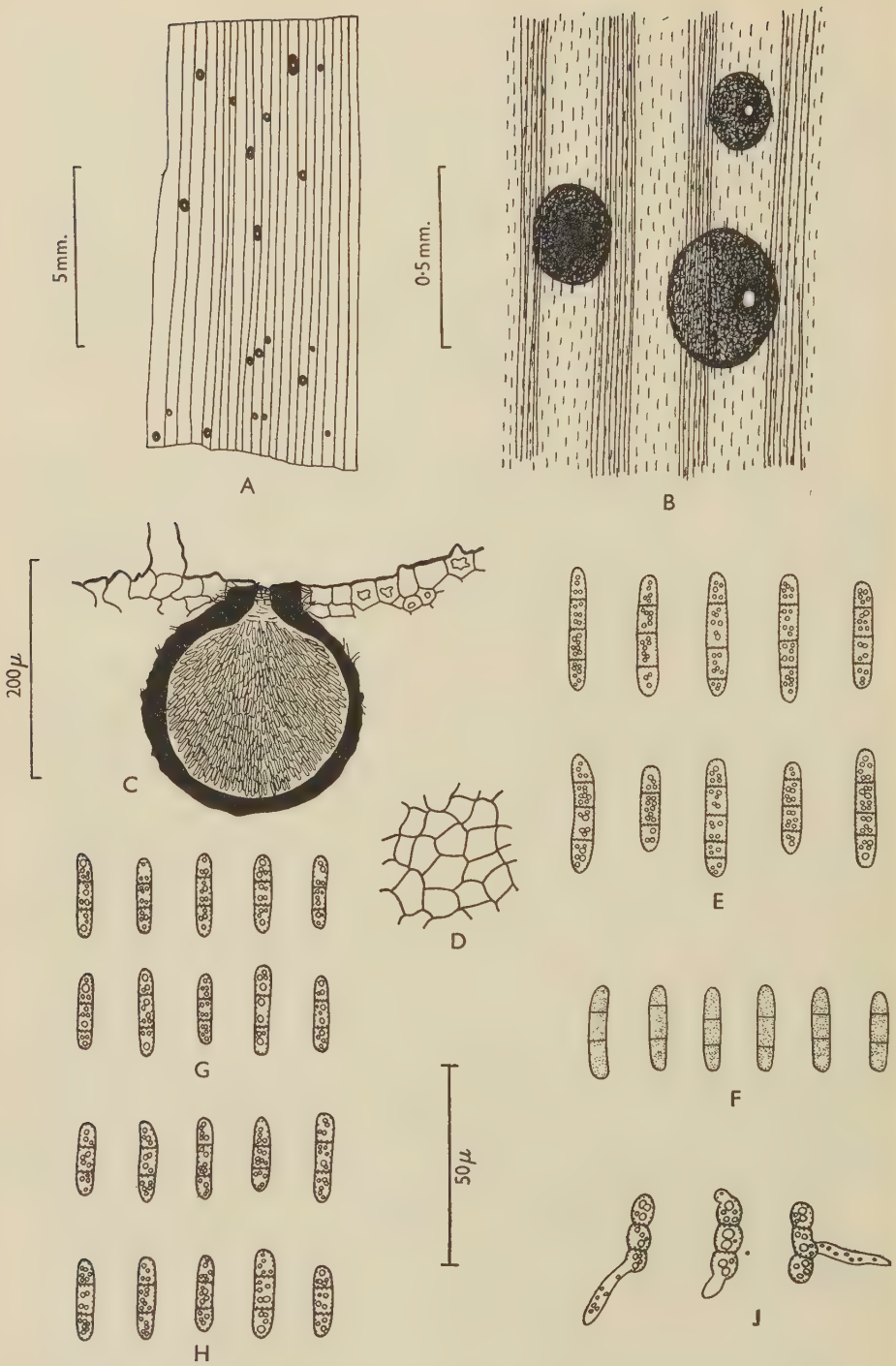

Fig. 5. A, part of leaf sheath of *Carex disticha* with pycnidia (× 5); B, part of same (× 50); C, vertical section of pycnidium on leaf sheath of *Carex hirta* (× 150); D, part of outer wall of pycnidium (× 555); E, ten random living spores from nature (× 555); F, six random spores of *Darluca macropus* (B. & Br.) Cooke ex. herb. Plowright in British Museum (Natural History) mounted in lactophenol. G, ten random living spores from pycnidia produced on re-infected *Carex riparia* (× 555); H, ten random living spores from pycnidia formed on 2% malt agar plate in light (× 555); J, same germinating after 12 hr. in water at room temperature.

Of the smaller spored species of *Stagonospora* recorded on *Carex*, *S. vitensis* Unam. appears to be the most appropriate name for this fungus. The spores are too narrow to belong to *S. strictae* Ell. & Ev. or *S. jaapii* Died. It has been impossible so far to obtain type or other named material of *S. vitensis* and no figures were published with the original description (Unamuno, 1929*a*). Unamuno gives a lower spore length range (15·5–24·6 μ) but the light colour and small size of the pycnidia (77·5 μ diameter) together with the apparent absence of ostioles strongly suggests that his material was not fully developed. In other respects this fungus fits Unamuno's description well and it seems reasonable to adopt his specific name. The only fungus recorded on *Carex*, with which this is likely to be confused, is *S. caricinella* Brun., but as previously indicated the latter has smaller pycnidia and the spores in nature are, on the average, smaller, have acute ends and tend to be constricted at the septa even when freshly released. These two species have been mistakenly 'lumped' under the name *Hendersonia macropus* B. & Br. A specimen of *Darluca macropus* (B. & Br.) Cooke from Plowright's herbarium at the British Museum (Natural History) has been examined. Spores from this material (Fig. 5 F) are very similar to those of *S. vitensis*, their slightly smaller dimensions being almost certainly due to the effects of drying.

The spores germinate readily in water at room temperature (Fig. 5 J), producing a single germ tube from each cell of the spore. On 2 % malt agar growth is rapid, giving a non-staling colony which is circular with a uniform but ragged edge due to the wide spacing of the leading hyphae. The aerial mycelium is uniform, densely cottony, usually Pale Olive-Grey to Dark Olive-Grey though one spore isolate gave an almost white colony. The colony has a characteristic speckled appearance centrally, due to the presence of numerous, small lighter coloured knots of hyphae on the surface of the aerial mycelium. In reverse the young colony is black centrally grading through Dark Greyish Olive to Pale Smoke Grey at the edge.

On oatmeal agar and on potato-dextrose agar the growth rate and the appearance of the colony are much the same as on 2 % malt agar but the aerial mycelium is more felted and the margin more even. Three-week-old cultures on all three media are similar with a uniform Olive-Grey to Deep Olive-Grey aerial mycelium. Reverse is Dark Olive-Grey to Olivaceous Black.

Fruiting is strikingly affected by light. Colonies grown on oatmeal agar at 20° C. in a light incubator (approx. 100 f.c.) for 15 days produce many pycnidia whereas parallel cultures grown in the dark produce only a few. When first isolated pycnidia are produced in abundance on 2 % malt agar in light. After 10 months' storage on 2 % malt agar at 10–15° C. this capacity is greatly diminished though many pycnidia are still formed on oatmeal or potato-dextrose agar.

Growth on sterilized *Carex* leaves produces a dense Light Olive-Grey to Dark Olive Grey surface mycelium. Beneath this numerous pycnidia are found. These are typical in appearance and position, but spore liberation is prevented by the surface mat of mycelium.

Spores produced in culture (Fig. 5 G, H) are similar to those from

nature though smaller. Dimensions of 100 spores taken at random from two different cultures are as follows:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
2% malt agar	17·0–25·0	20·2	1·52	3·5–5·0	4·3
Re-infected *Carex*	18·0–25·0	20·7	1·52	3·5–4·5	4·0

Specimens of this species have been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 58656), Kew Herbarium and the British Museum (Natural History).

Stagonospora caricis (Oud.) Sacc.

S. caricis has been found in November on dead leaves of *Carex otrubae* and dead leaf tips of *C. acutiformis* and in April on the lower parts of the leaves of *C. pseudocyperus*. Pycnidia on *C. pseudocyperus* were growing about 30 cm. below water-level. The following description is based on this submerged material.

The pycnidia are mostly beneath the abaxial side of the dead leaf, scattered and not confined to definite spots (Fig. 6 A, B). They are small, spherical with a diameter of 110–220 μ (average of 50, 150 μ) and lie completely beneath the surface at which they open by a small pore 10–15 μ in diameter (Fig. 6 C, D). The pycnidial wall is 12–18 μ thick and consists of several layers of thin cells the outermost of which are irregular in shape, 5–15 μ across (Fig. 6 E) and have a Tawny-Olive colour when viewed by transmitted light under low power of the microscope.

The spores (Fig. 6 F) are fusiform, straight or slightly curved and have sub-acute or rounded ends. They are colourless with thin smooth walls. Typically five septa are present but smaller spores have fewer and the largest ones are 7-septate. Each cell contains a number of refractive oil drops. Length is variable but the majority of spores are 32–37 μ long. The dimensions of 100 spores from *C. pseudocyperus* taken at random are: length, 25·0–45·0 (mean, 34·3) μ (standard deviation, 3·0); width, 5·0–8·0 (mean, 6·5) μ.

There can be little doubt that this is the same species that was originally described as *Hendersonia caricis* Oud. on *C. muricata* from Holland (Oudemans, 1873). The present material fits this description except in spore dimensions which are given as 25–50 μ × 4–5 μ. However, Oudeman's figure of six spores gives dimensions of 18–23 μ × 3·5–5 μ at the scale quoted. Though this indicates an error in magnification of the drawing the proportion of width to length (1 : 4·8) is lower than with the measurements given (1 : 8·3) and is more in accord with the ratio in my specimen (1 : 5·3). Later authors ascribe a higher width range to the spores. For example, Grove states '. . . spores . . . generally 5-septate, 25–40 × 4–6 μ, but some spores 7-septate and up to 40–50 × 6–8 μ' (Grove, 1935).

Four other species of *Stagonospora* fall within the same range of spore length as my material. Of these *S. caricicola* Unam. (25–35 × 5·7–6·5 μ,

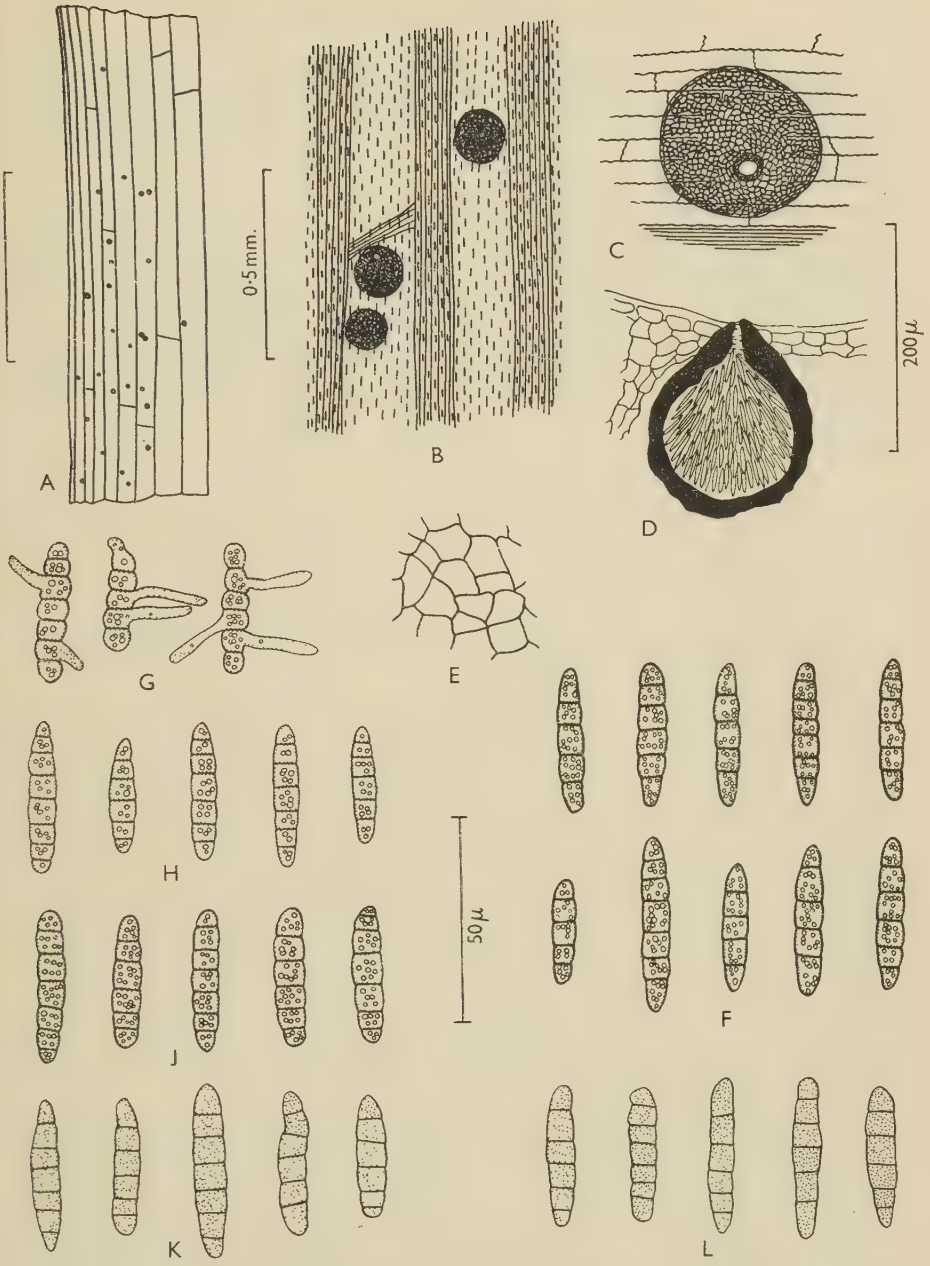

Fig. 6. A, part of leaf of *Carex pseudocyperus* with pycnidia (× 5); B, part of same (× 50); C, surface view of single pycnidium (× 150); D, vertical section of pycnidium (× 150); E, part of outer wall of pycnidium (× 555); F, ten random living spores from nature (× 555); G, germinating spores after 18 hr. in water at room temperature (× 555); H, five random living spores from pycnidia produced on 2 % malt agar (× 555); J, five random spores from pycnidia produced on re-infected *Carex riparia* leaves (× 555); K, five random spores mounted in lactophenol and cotton blue of *Stagonospora caricis* (Oud.) Sacc. from material on *Carex riparia* at Kew Herbarium (× 555); L, five random spores mounted in lactophenol and cotton blue of syntype material of *Stagonospora asturica* Unam. (× 555).

(1) 2 (3) septate) (Unamuno, 1931) has fewer septa as has also the wide-spored *S. hiratsukana* Togashi (30–45 × 7–10 μ, (1) 3 (4) septate) (Togashi, 1936), and the spores of *S. caricicola* Baudyš (29–46 × 2·5–3 μ, 5- to 7-septate) (Baudys, 1916) as described and figured are much narrower than in this material. Unamuno has described *S. asturica* from Spain which he emphasizes is distinct from *S. caricis* (Oud.) Sacc. (Unamuno, 1929*b*). However, the description and figures agree with those of *S. caricis* though the spores (30·5–42·5 × 5–7 μ, (4) 6 (7) septate) are slightly wider as in the present material. Syntype material of *S. asturica* has been examined and the spores (Fig. 6 L) are seen to be very similar to those of *S. caricis*. For comparison herbarium material of the latter on *Carex riparia* (J. W. Ellis, Cheshire, 18 April 1914) at Kew Herbarium has been examined (Fig. 6 K). Dimensions of 50 spores taken at random from each mounted in lactophenol are:

	Length (μ)		Width (μ)		
Source	Range	Mean	Range	Mean	Septa
S. asturica Unam.	28·0–42·0	34·2	5·5–8·5	6·8	(4) 5–6 (7)
S. caricis (Oud.) Sacc.	28·0–44·0	35·1	5·5–8·0	6·9	(4) 5–6 (7)

Thus it is very probable that *S. asturica* Unam. is the same species as *S. caricis* (Oud.) Sacc. but as type material of the latter has not been examined this cannot be confirmed.

In water at room temperature the spores readily germinate (Fig. 6 G). On 2 % malt agar a circular, non-staling colony with a ragged edge is formed. The aerial mycelium is sparse, though denser centrally, Dawn Grey to Pearl Grey in colour. The marginal 7 mm. is without aerial hyphae. The reverse is black centrally grading to Sepia and then to Tawny-Olive about 10 mm. from the edge.

The appearance is similar on potato-dextrose agar but the edge is more ragged and the aerial mycelium uneven with whitish patches. The reverse is black in the centre. On oatmeal agar the edge of the colony is more uniform and the marginal zone is only 4 mm. wide.

Old colonies have a uniform aerial mycelium Pallid Mouse Grey to Deep Mouse Grey in colour with the reverse uniformly black. When first isolated a few pycnidia are formed on malt agar, but after 6 months in culture none are produced.

On re-infected sterile *C. riparia* leaves a uniform, felted white to Deep Mouse Grey mycelium is formed. When first isolated many typical pycnidia were formed but after 18 months in culture fruiting under these conditions has been reduced to a very low level.

Spores produced in culture (Fig. 6 H, J) are similar to those in nature, though from 2% malt agar cultures they are slightly smaller and less variable in size. Dimensions of 100 spores taken at random from two cultures are:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
2 % malt agar	24·0–35·0	30·7	2·3	4·5–6·5	5·5
Sterilized *Carex*	27·0–44·0	34·0	3·0	5·0–8·0	6·4

Material of this fungus on *Carex pseudocyperus* has been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 59582), Kew Herbarium and the British Museum (Natural History).

Stagonospora paludosa (Sacc. & Speg.) Sacc.

Stagonospora paludosa is undoubtedly the commonest species occurring on the waterside sedges. It has been found many times between September and April, especially on *Carex riparia*, *C. acutiformis* and *C. pseudocyperus*. Often pycnidia containing healthy spores are present on submerged *in situ* leaf bases and also on dead flowering stems and leaf tips which bend over into the water. The fungus has also been found on quite dry papery pieces of leaves. On one occasion (November, 1953) it was found on the dead submerged leaves of a plant of *Acorus calamus* which was present in a dense community of *C. acutiformis* on which this fungus was also growing.

The pycnidia (Fig. 7 A, B) on *Carex* and *Acorus* are amphigenous and often widely scattered though sometimes loosely gregarious. They are brown to almost black in colour and are completely immersed in the surface tissues of the leaf. The pycnidia are globose with a diameter of 100–200 μ (average of 50, 170 μ) and a pore diameter of 20–30 μ. The pycnidial wall is thin (Fig. 7 E), consisting of few layers of thin cells. The cells of the outer layer are irregular in shape, 5–12 μ across, have dark brown walls and sometimes contain one or two small oil drops (Fig. 7 C). By transmitted light under low power of the microscope they are Dresden Brown in colour, darker round the pore.

Sectioning material from nature has revealed one old, apparently discharged pycnidium containing within it a younger one with spores (Fig. 7 D). The ostiole is closed and the young pycnidium is attached at the upper region and projects into the empty cavity of the discharged pycnidium. This phenomenon has been found occasionally in culture of this and other species and has been recorded in *S. asturica* Unam. in nature (Unamuno, 1929*b*).

The spores (Fig. 7 F, G) are fusiform, straight or sometimes curved with acute ends, though in some the proximal end tends to be more rounded. They are hyaline with smooth walls slightly constricted at the septa. Most have 6–8 obvious septa but some have more, those at the ends being delicate and difficult to see in the living spores. Healthy spores have many small oil drops per cell. Spores removed from old closed pycnidia both from culture and in nature often have one or two large oil drops per cell (Fig. 7 H). These spores are still capable of germination when mounted in water (Fig. 7 J).

Dimensions of 100 spores taken at random from three collections are:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Carex acutiformis	41·0–62·0	53·6	4·43	8·0–11·0	9·5
C. riparia	44·0–63·0	52·8	4·82	8·0–11·0	9·8
Acorus calamus	46·0–67·0	56·2	4·80	8·0–11·0	9·2

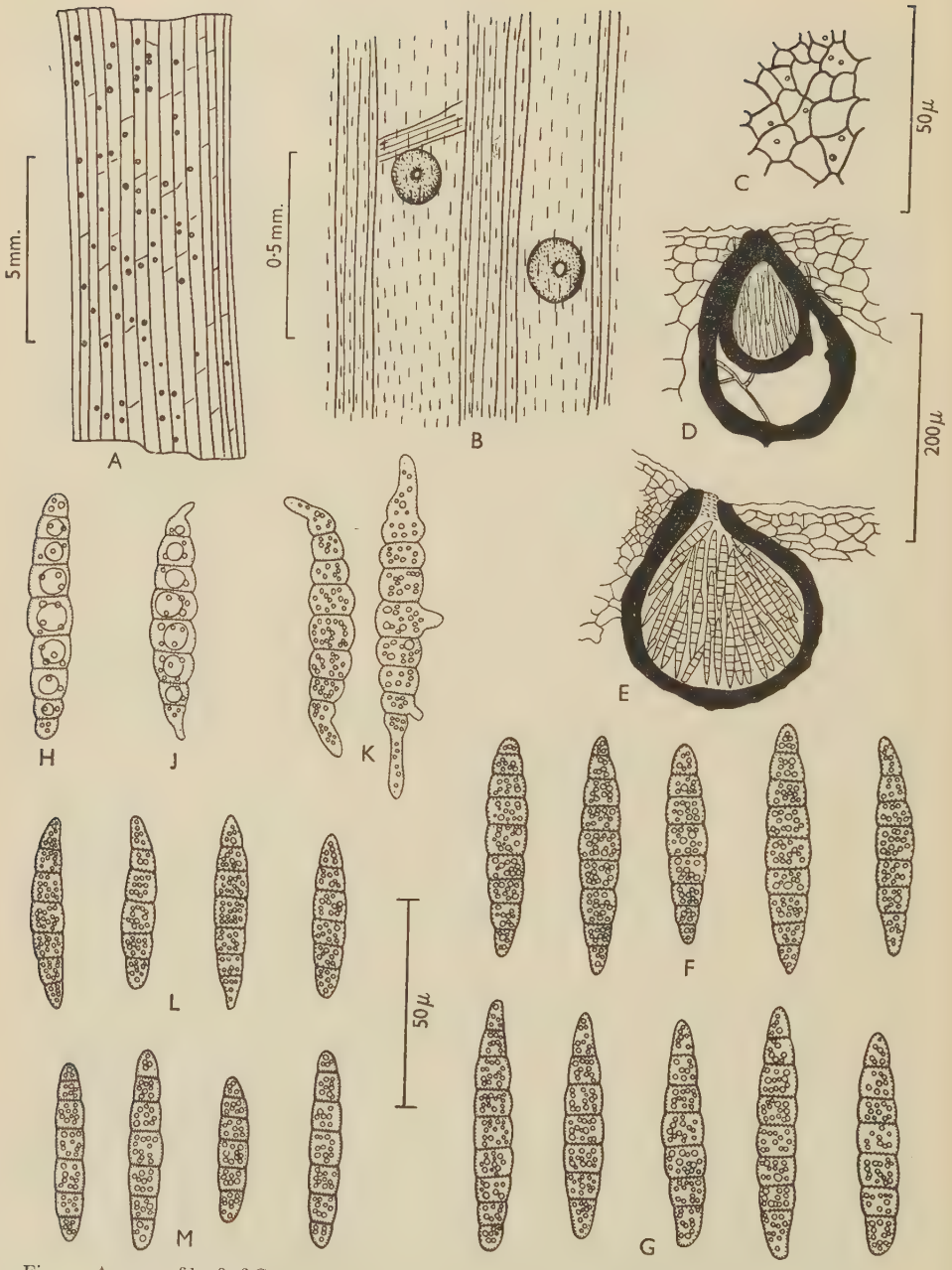

Fig. 7. A, part of leaf of *Carex acutiformis* with pycnidia (× 5); B, part of same (× 50); C, part of outer wall of pycnidium (× 555); D, vertical section of old pycnidium with young one inside, from naturally occurring material on *Carex acutiformis* (× 150); F, vertical section of normal mature pycnidium on *Carex acutiformis* (× 150); E, five random living spores from nature from *Carex acutiformis* (× 555); G, five random living spores from nature from *Acorus calamus* (× 555); H, spore from old pycnidium on dry leaf base of *Carex pseudocyperus* (× 555); J, similar spore after eight hours in water at room temperature (× 555); K, normal spores after 12 hr. in water at room temperature (× 555); L, four random living spores from re-infected *Carex riparia* (× 555); M, four random living spores from oatmeal agar plate (× 555).

There can be little doubt that this is the fungus originally described as *Hendersonia paludosa* Sacc. & Speg. on dead leaves of *Carex riparia* in Italy (Saccardo, 1879). Saccardo's exciccata material at Kew Herbarium and the British Museum (Natural History) has been examined, but this fungus has not been found. The present material agrees well with his description, though the ranges of dimensions found are greater than those quoted by him (pycnidia 100–120 μ; spores 50–60 × 8–10 μ).

Other British specimens at Kew have been examined, viz. on *C. ampullacea*, Aberdeen (J. W. H. Trail) and on *C. riparia*, Cheshire (J. W. Ellis) and found to agree with the present fungus. Though the spores of Trail's specimen are smaller (42–52 × 8 μ) they fall into the ranges quoted above and definitely belong to *S. paludosa*.

This fungus is not likely to be confused with any of the species on *Carex* dealt with here except *S. gigaspora* the average spore size of which is greater. *S. paludosa* (Sacc. & Speg.) Sacc. var. *caricis-pendulae* Unam. has been described on *C. pendula* from Spain (Unamuno, 1929*a*). Examination of syntype material of this suggests that it is in fact *S. gigaspora* (see below).

The spores germinate rapidly in water at room temperature (Fig. 7 K). Germ tubes are usually produced from the end cells of the spore first. On malt agar a slow-growing, non-staling colony is formed which may be circular or produce slower growing sectors. The edge is very ragged due to the wide spacing and varying length of the leading hyphae. The aerial mycelium is uniform, dense, felty, Hathi Grey centrally to Pearl Grey near the edge. The reverse is black centrally grading irregularly to Deep Olive-Grey with the marginal 5 mm. white. On both potato-dextrose agar and oatmeal agar the colonies are similar but the growth rate is slightly greater on potato-dextrose agar.

Old colonies are uniformly Storm Grey to white, black in reverse. Fruiting is poor on the agar media used, a few pycnidia being found only on the colonies of fresh isolates.

On sterilized *Carex* leaves the mycelium is sparse and many typical pycnidia with spores are formed. The spores in culture (Fig. 7 L, M) are very much smaller than those found in nature. Dimensions of 100 spores taken at random from two cultures are:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Oatmeal agar	35·0–50·0	42·8	3·6	7·0–10·0	8·1
Re-infected *Carex*	37·0–50·0	42·0	2·7	7·5–10·0	8·6

Specimens of *S. paludosa* have been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 58655, 58657), Kew Herbarium and the British Museum (Natural History).

Stagonospora gigaspora (Niessl) Sacc.

I have not found this species in the living condition, but, for completeness, material collected by E. A. and M. B. Ellis from Wheatfen Broad,

Norfolk and deposited in the Commonwealth Mycological Institute (Herb. I.M.I. 13,695, dated 4 April 1947) has been examined.

The fungus is on the dead upper parts of leaves of *Carex riparia*. The pycnidia (Fig. 8 A, B) are amphigenous, loosely gregarious and completely immersed. They are spherical, 150–240 μ in diameter (average of 50,195 μ) and project into the cavity of the leaf. Each is attached to the leaf tissue by a short neck which opens at the surface by a pore 20–25 μ in diameter (Fig. 8 C). When viewed from above by transmitted light only the dark cells round the pore are clearly seen and the outline of the pycnidium is hardly discernible. The wall is 27–40 μ thick and consists of several layers of densely packed cells. The cells of the outer layer are irregular in shape and have undulating walls (Fig. 8 D). They are 3–12 μ across. When crushed and viewed by transmitted light the wall is Mummy Brown in colour.

The spores (Fig. 8 E) are variable in shape and size, but most are fusiform, straight and have more or less acute ends. Others may be cylindrical, slightly curved or have rounded ends. There are 6–9 obvious septa at which the smooth outer wall is constricted. The spores are hyaline with several oil drops per cell.

The dimensions of 100 spores from *C. riparia* taken at random, mounted in lacto-phenol, are: length, 58·0–84·0 (mean, 68·1) μ (standard deviation, 4·42); width, 10·0–14·0 (mean, 11·4) μ.

This agrees well with the original description of *Hendersonia gigaspora* Niessl (Niessl, 1883).

Syntype material of this fungus ex. herb. Rabenhorst in the British Museum (Natural History) has been examined. The spores (Fig. 8 F) are similar to those of the British material but tend to be on the average slightly larger and have fewer septa. Dimensions of fifteen spores measured in lacto-phenol were 60–84 × 12–14 μ (average, 74·5 × 12·6 μ).

Material of *S. paludosa* (Sacc. & Speg.) Sacc. var. *caricis-pendulae* Unam. on *C. pendula* from Spain has been examined. The original description of this (Unamuno, 1929*a*) is correct but the specimen is indistinguishable from the British material of *S. gigaspora*. This is shown by the spores (Fig. 8 G). Dimensions of fifty spores measured in lacto-phenol were 56–77 × 9–13 μ (average, 67·1 × 11·1 μ). Unamuno clearly indicates how his fungus differs from the type of *S. paludosa* but he was apparently unaware of the species described by Niessl.

Stagonospora anglica sp.nov.

Pycnidia amphigena, dispersa, nigra, immersa, ellipsoide, magna 310–500 × 170–340 μ. Sporulae fusiformes, plerumque rectae, in extremis rotundae aut obtusae, 5–8-septatae, paulum constrictae ad septa, multis parvis olei guttis praesentibus, hyalinae, 68·0–109·0 × 14·0–18·0 μ.

Hab. In mortuis foliis *Caricis acutiformis*, circa Hounslow, Middlesex, in Anglia, 28 November 1954 Typus: Herb. I.M.I. 59583.

A small collection of this fungus was made in November 1954 on dead saturated leaves of *Carex acutiformis* in a cutting off the River Crane, Hounslow, Middlesex.

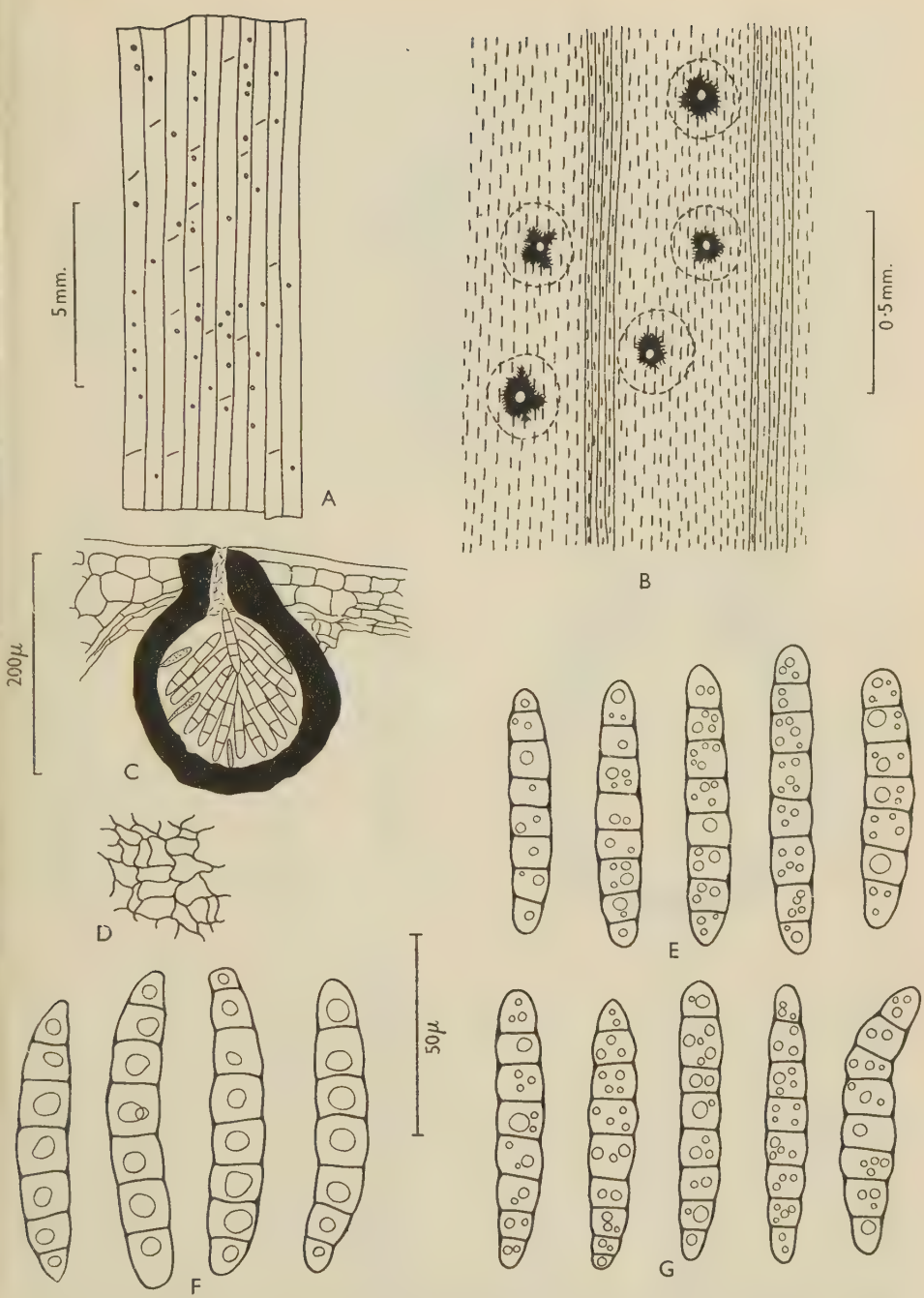

Fig. 8. A, part of *Carex riparia* leaf with pycnidia (× 5); B, part of same (× 50); C, vertical section of pycnidium (× 150); D, part of outer wall of pycnidium (× 555); E, random spores of *Stagonospora gigaspora* from pycnidia on *Carex riparia* mounted in lactophenol (× 555); F, four random spores of syntype material of *Hendersonia gigaspora* Niessl ex herb. Rabenhorst in British Museum (Natural History) (× 555); G, five random spores of syntype material of *Stagonospora paludosa* (Sacc. & Speg.) Sacc. var. *caricis-pendulae* Unam.

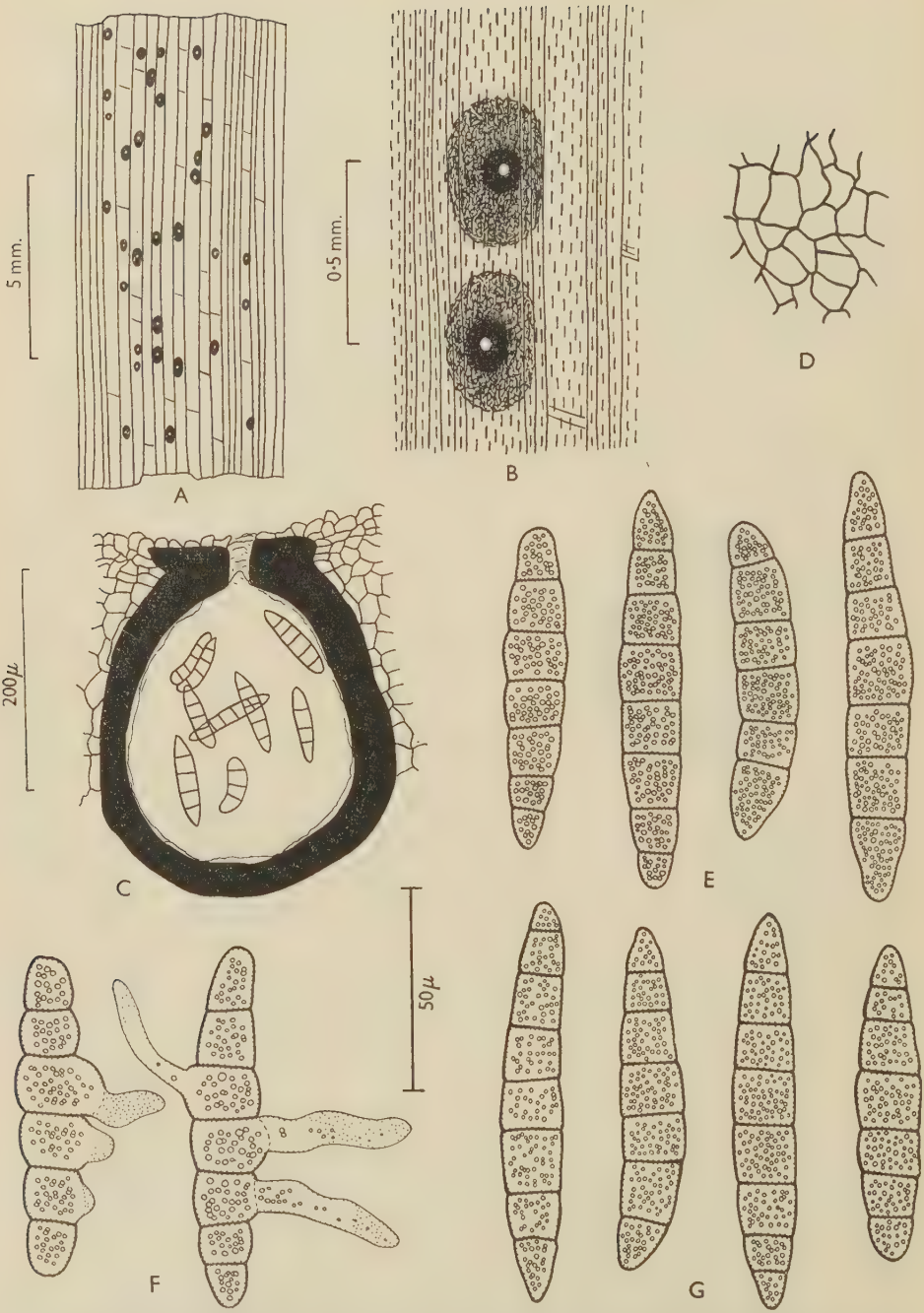

Fig. 9. A, part of leaf of *Carex acutiformis* with pycnidia (× 5); B, part of same (× 50); C, vertical section of pycnidium (× 150); D, part of outer wall of pycnidium (× 555); F, germinating spores after 24 hr. in water at room temperature (× 555); E, four random living spores from nature from *C. acutiformis* (× 555); G, four random living spores from re-infected *C. riparia* (× 555).

The pycnidia (Fig. 9 A, B) are amphigenous and, though more or less scattered, tend to occupy light brown regions of the dead leaf. They are large and mostly ellipsoidal with the long axes parallel to the vein of the leaf. They measure 310–500 × 170–340 μ (average of 50, 382 × 266 μ). The ostiole is 20–30 μ across and is situated in the centre of a depressed apical disk (Fig. 9 C). The pycnidial wall is 20–40 μ thick and composed of several layers of flattened cells. The outer layer (Fig. 9 D) consists of cells variable in shape 5–15 μ across. When viewed by transmitted light under low power of the microscope the wall is Olive-Brown in colour.

The spores (Fig. 9 E) are large, fusiform, mostly straight and have rounded or obtuse ends. The smooth outer walls are slightly constricted at the septa of which 5–8 are present. The spores are hyaline with many small oil drops in each cell. The dimensions of 100 living spores from *C. acutiformis* taken at random are: length, 68·0–109·0 (mean 87·5) μ (standard deviation 10·65); width, 14·0–18·0 (mean, 16·0) μ.

The largest spored species of *Stagonospora* recorded on *Carex* is *S. gigaspora*. Though the length ranges overlap slightly the spores of this species are mostly larger and much wider than those of *S. gigaspora*. *S. megistospora* Rost. described on *Scirpus maritimus* from Denmark (Rostrup, 1916) has not been examined, but the spores are described as 118–137 × 14–17 μ, 6- to 10-septate, which are much larger than those of *S. anglica*. Three other fungi, occurring on Monocotyledons, but not recorded from Britain, have been examined in Kew Herbarium, but the spores of these are too narrow to be considered the same as those of the present material. These are: *S. macrospora* Sacc. & Roum. on Gramineae, France (85–95 × 12–14 μ) (Saccardo, 1884), *S. macrospora* (Dur. & Mont.) Sacc. on *Agave americana*, Sicily (100 × 10–14 μ) (Saccardo, 1906) and *S. gigantea* Heald & Wolf, parasitic on *Agave americana*, N. America (72–118 × 13–15 μ) (Saccardo, 1931). Perusal of the literature, and, when possible, examination of herbarium specimens have failed to suggest a suitable name for this fungus which is, therefore, described as a new species.

The spores germinate in water at room temperature. In all those examined the first germ tubes are formed from the central cells of the spore (Fig. 9 F). On 2 % malt agar growth is rapid and a circular, non-staling colony with a ragged edge is formed. The aerial mycelium is abundant but loose, cottony and uniformly white. The reverse is white.

On potato-dextrose agar and oatmeal agar similar colonies are found though in the latter the edge is more uniform with the leading hyphae close together. Old colonies have a uniformly white aerial mycelium and black sclerotial masses scattered in the agar. So far fruiting has not been observed on the three media used.

On sterilized, re-infected *Carex* leaves a sparse white surface mycelium is formed. Many pycnidia identical with those in nature are produced and contain typical 5- to 9-septate spores though the mean width is slightly less. Dimensions of 100 random spores from re-infected *Carex* are: length, 70·0–113·0 (mean, 88·0) μ (standard deviation, 7·65); width, 13·5–18·5 (mean, 14·7) μ.

Part of the type collection has also been deposited in Herb. Kew.

Discussion

Limited collection, during the past 2 years, of the pycnidial fungi described in this paper indicates that they are very common, though past British records are scanty. Though present throughout the year, they are most abundant during the winter months on the dead leaves of the previous season's growth. They have only been found on brown dead leaves and there is no evidence of parasitic activity.

Of the many species of *Carex* in this country only about ten of the commoner ones have been examined for these fungi. There seems little doubt, however, that they are widespread on members of the genus, particularly on the marsh species. Repeated collection of Sphaeropsidales on other marsh and reed-swamp plants has occasionally revealed the fungi dealt with here on plants other than sedges, e.g. *Stagonospora caricinella* on *Sparganium ramosum*, and *S. paludosa* on *Acorus calamus*. Such cases have been rare and only found where the host plant has been growing in a dense community of sedges on which the fungus was abundant. Thus it appears that these fungi are largely restricted to the genus *Carex*.

Other Sphaeropsidales, very similar to those considered here, have been found on other host genera. However, when such a species is isolated and compared with an apparently similar one on *Carex* it is usually found to behave quite differently in culture. For example, *Stagonospora typhoidearum* on *Typha* spp. is similar to *S. vitensis* in nature but their cultural features are very distinct.

These observations, therefore, support the practice which has often been followed when dealing with the taxonomy of these pycnidial fungi, of naming distinct species on the basis of occurrence on different host genera. On the other hand, the fact that they have occasionally been found on other hosts in nature, emphasizes that the host range is not necessarily as narrow as this. A specimen may not fit any described species on a given host in the literature or in herbaria but may, none the less, be a clearly defined species normally found on other host plants.

In general pycnidial characters are of little use in identifying these fungi. The pycnidia of *Stagonospora anglica* are relatively large and, as they develop, lateral expansion is prevented by the adjacent veins of the leaf so that at maturity they are ellipsoidal (Fig. 9 B). This is seen to a lesser extent in *S. vitensis* (Fig. 5 B). The pycnidia of the other species dealt with show considerable overlap in size ranges and they are mostly spherical as there is little resistance to their uniform enlargement by the surrounding leaf tissue. However, features of the pycnidia are useful in separating *Diplodina teretiuscula* and *Ascochyta sodalis* which are often closely associated and have very similar spores. The pycnidia of *Diplodina teretiuscula* are larger (Fig. 2 D) with small wall cells (Fig. 2 C), whereas those of *Ascochyta sodalis* are much smaller (Fig. 3 D) and have large wall cells, many containing conspicuous oil drops (Fig. 3 G).

Commonly pycnidia containing healthy spores occur well below water-level and on saturated pieces of leaf. However, closed pycnidia containing living spores capable of germination have been observed in some species (e.g. *Stagonospora paludosa*) on very dry leaves. Young and freshly matured

spores normally have a number of small oil drops in each cell. In old spores, retained within pycnidia for some time especially under dry conditions, the oil drops tend to coalesce to form one large one in each cell (Fig. 7 H). These spores are often still viable. Coalescence of the oil to a single large drop in each cell is also a feature of dead spores. In taxonomic descriptions reference is frequently made to the presence of one oil drop or guttule in each cell. Obviously the spores of herbarium or dead material are being described and not those of living specimens.

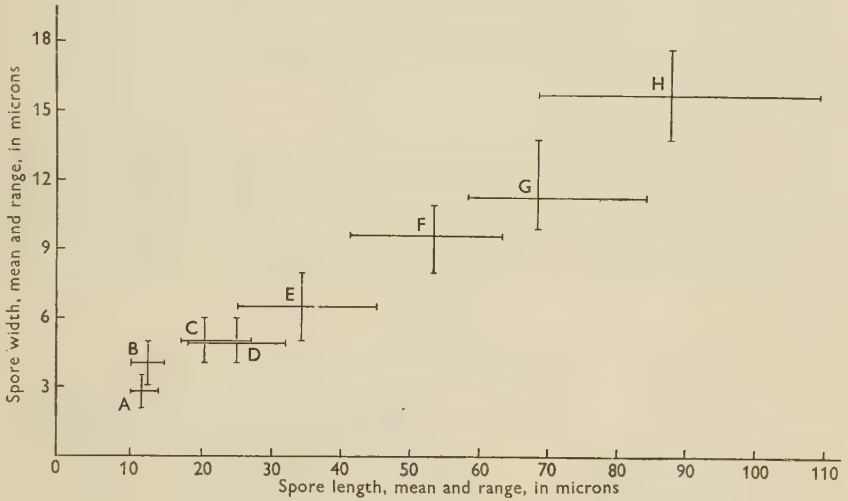

Fig. 10. Comparison of length and width (ranges and means) of living spores in water as found in nature on dead leaves of *Carex* spp. A, *Diplodina teretiuscula* (200 spores); B, *Ascochyta sodalis* (200 spores); C, *Stagonospora caricinella* (200 spores); D, *S. vitensis* (300 spores); E, *S. caricis* (100 spores); F, *S. paludosa* (200 spores); G, *S. gigaspora* (100 spores from herbarium material mounted in lactophenol); H, *Stagonospora anglica* (100 spores).

Spore dimensions represent the most important criteria for separating these species. This is especially true of those with small spores where considerable overlap in length and width occurs (Fig. 10). It is essential that a number of spores from a number of pycnidia of fresh, healthy material should be measured under uniform conditions. When this is done there is little difficulty in making a correct identification.

Further difficulty is encountered when dealing with spore size in culture. With the semi-natural media used and even on the natural substratum in the laboratory it is found that spore size often differs considerably from that of the original material from which the isolate was made. In general it appears that the smaller-spored species tend to produce larger spores in culture (e.g. *S. caricinella*), whereas the larger-spored species produce smaller ones in culture (e.g. *S. paludosa*). In these two examples there is also a greater variability of spore size in culture than under natural conditions. The largest spores of *S. caricinella* (Fig. 4 H) and the smallest spores of *S. paludosa* (Fig. 7 M) in culture are very similar and are indistinguishable

from those of *S. caricis* (Fig. 6 F). Thus difficulties may arise in attempts to identify an isolate only on the size of spores produced in culture.

When *Asochyta sodalis* is grown on oatmeal agar, perithecia, referable to the genus *Mycosphaerella*, are produced. This conforms with the connexion established between *Ascochyta pinodes* Jones and *Mycosphaerella pinodes* (Berk. & Blox.) Stone (Sattar, 1934). A *Leptosphaeria* stage is produced when *Stagonospora caricinella* is grown on sterilized *Carex* leaves. It has been shown that some *Leptosphaeria* spp. have imperfect stages, e.g. *L. nigrans* and *Phaeoseptoria* spp. (Hughes, 1949), but as far as is known no record of the connexion between a *Stagonospora* and a *Leptosphaeria* has been published. Numerous Pyrenomycetes occur on dead leaves of sedges, and several of these have been cultured but none has produced an imperfect stage or colony similar to those described. Further work on this problem is in progress.

As far as is known none of these fungi have been isolated in pure culture before. A comparison of their colony appearance on agar indicates a general similarity, all having a white to dark grey, more or less fluffy aerial mycelium. Some can be clearly distinguished by comparison of growth rates (Table 1), whereas others show definite fruiting charac-

Table 1

Species	Increase in diameter on 2% malt agar at 20° C. in millimetres per 24 hr.		Combined average
Ascochyta sodalis	6·4*	5·4*	5·9
Diplodina teretiuscula	4·3	3·8	4·1
Stagonospora caricinella	10·1	10·0	10·1
S. vitensis	9·3	9·2	9·3
S. caricis	8·7	—	8·7
S. paludosa	7·1	6·2	6·7
S. anglica	10·9	10·7	10·8

* Each figure in the first two columns is the average daily increase in diameter of three colonies of a single spore isolate over a period of 3 days.

teristics in culture as indicated above. For example, *Stagonospora caricinella*, *S. caricis* and *S. paludosa* which tend to produce similar spores in the few pycnidia formed, have very different growth rates. *S. caricinella* and *S. vitensis* have similar growth rates, but the latter has a much denser and darker aerial mycelium and produces many pycnidia on oatmeal agar whereas the former has a thin, light-coloured mycelium and only produces a few pycnidia.

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REFERENCES

Allescher, A. (1901). *Rabenhorst's Kryptogamen-Flora* I. *Die Pilze*, Abt. **6**.

Baudys, E. (1916). Ein Beitrag zur Kenntnis der Mikromyceten in Böhmen. *Lotos*, **62**, 57.

Berkeley, M. J. & Broome, C. E. (1850). Notices of British Fungi. *Ann. Mag. nat. Hist.* **5**, 365–380.

Brunaud, P. (1893). Sphéropsidées nouvelles ou rare recoltées à Saintes-Porchaine, à Fouras et à Saintes (Char.-Infér.). *Bull. Soc. bot. Fr.* 2e Série, **15**, 221–225.

Clapham, A. R., Tutin, T. G. & Warburg, E. F. (1952). *Flora of the British Isles*. Cambridge University Press.

Cooke, M. C. (1871). *Handbook of British Fungi*, **1**. London.

Diedicke, H. (1915). *Kryptogamen-Flora der Mark Brandenburg, Pilze*, **7**, 553–554.

Ellis, J. B. & Everhart, B. M. (1893). New species of North American Fungi from various localities. *Proc. Acad. nat. Sci. Philad.* pp. 128–172.

Fuckel, K. W. G. L. (1869). *Symbolae Mycologicae Beiträge zur Kenntnis der Rheinischen Pilze*. Wiesbaden.

Grove, W. B. (1935). *British stem- and leaf-fungi*, **1**. Cambridge.

Hughes, S. J. (1949). The perithecia and pycnidia of *Leptosphaeria nigrans*. *Trans. Brit. mycol. Soc.* **32**, 63–68.

Migula, W. (1921). *Kryptogamen-Flora von Deutschland, Deutsch-Oesterreich, und der Schweiz*, III (**4**:**1**).

Niessl, G. V. (1883). Repertorium. Rabenhorstii Fungi europaei et extraeuropaei. Cent. XXX. cura Dr. G. Winter. *Hedwigia*, **22**, 180–183.

Oudemans, C. A. J. A. (1873). Matériaux pour la flore de la Neerlande, II. *Arch. néerl. Sci.* **8**, 343–416.

Ridgway, R. (1912). *Color standards and color nomenclature*. Washington, D.C.

Rostrup, O. (1916). Bidrag til Danmarks Svampeflora I. *Dansk. Bot. Ark.* **2**, 1–56.

Saccardo, P. A. (1879). Fungi veniti novi v. critici. Series VIII. *Michelia*, **1**, 351–355.

Saccardo, P. A. (1884). *Sylloge Fungorum*, **3**.

Saccardo, P. A. (1892). *Sylloge Fungorum*, **10**.

Saccardo, P. A. (1899). *Sylloge Fungorum*, **14**.

Saccardo, P. A. (1906). *Sylloge Fungorum*, **18**.

Saccardo, P. A. (1931). *Sylloge Fungorum*, **25**.

Sattar, A. (1934). A comparative study of the fungi associated with blight diseases of certain cultivated leguminous plants. *Trans. Brit. mycol. Soc.* **18**, 276–301.

Togashi, K. (1936). A contribution to the parasitic-fungus flora of Mt. Iwate, Iwate Prefecture. *Bull. Coll. Agric. For. Morioka.* **22**, 41.

Unamuno, L. M. (1929*a*). Hongos microscópicos de los alrededores de la Vid (Burgos). *Bol. Soc. esp. Hist. nat.* **29**, 387–402.

Unamuno, L. M. (1929*b*). Nuevos datos para el estudio de la flora micológica de los alrededores de Llanes (Asturias). *Mem. Soc. esp. Hist. nat.* **15**, 345–354.

Unamuno, L. M. (1931). Contribución al estudio de los hongos microscópicos de la provincia de Salamanca. *Bol. Soc. esp. Hist. nat.* **31**, 86–96.

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INITIATION OF THE DICARYON IN *PUCCINIA PENNISETI* ZIM.

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(With 5 Text-figures)

The initiation of the dicaryon in *Puccinia penniseti* takes place by 'cell-fusions', a method which is constant for this rust.

Since the classical cytological investigations on the rust fungi by Blackman (1904) and Christman (1905), numerous papers have been published on this and related aspects of these fungi by workers who have found the mode of initiation of the dicaryon to be either of the 'Blackman Type' (nuclear migrations) or the 'Christman Type' (cell fusions).

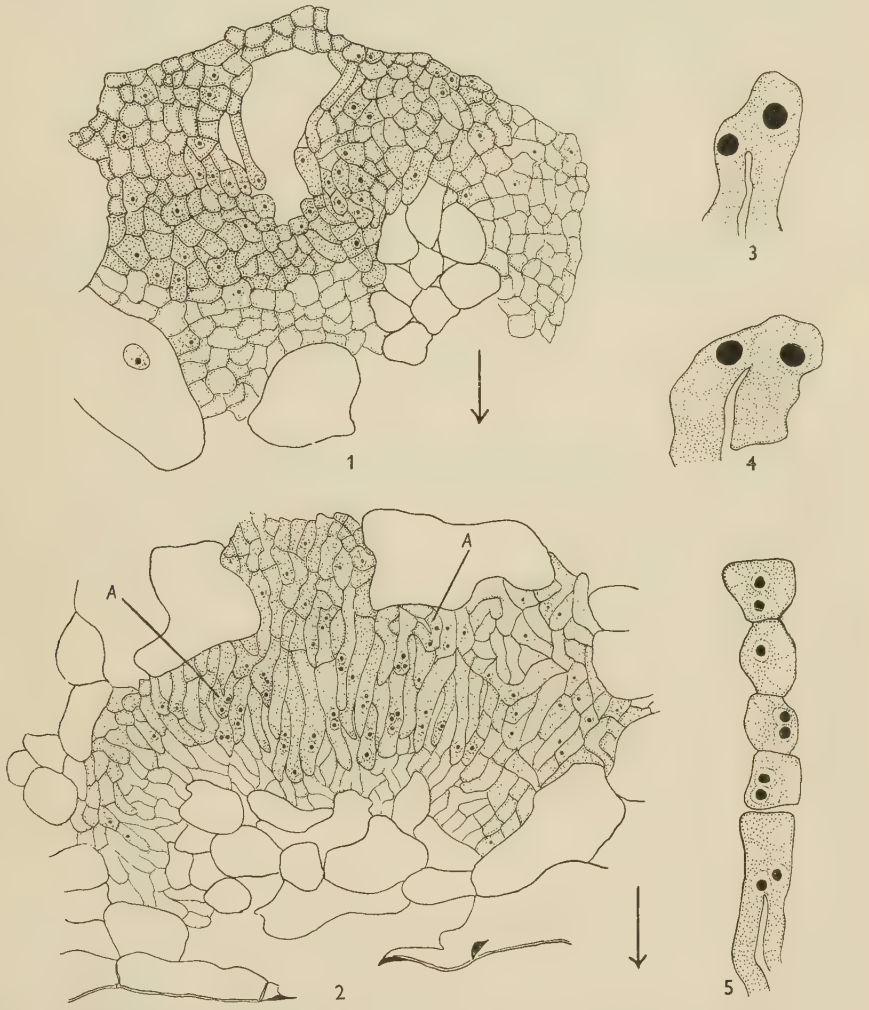
During the course of studies on the cytology, nuclear behaviour and developmental processes of the various spore-forms of *Puccinia penniseti* Zim., the leaf rust of bajra (*Pennisetum typhoideum*), the writer had occasion to make observations on the manner in which the dicaryon is attained in this fungus and the results are presented in this note.

The pycnial and aecial stages of the rust were secured by experimental inoculations of the young leaves of brinjal (*Solanum melongena*), the alternate host of this rust, with basidiospores. Leaves were fixed at intervals in formol-acetic-alcohol and microtome sections, 6–10 μ in thickness, were stained in Heidenhain's haematoxylin and counter-stained with orange G in clove oil. Best results with various stages of the process of dicaryotization were obtained with material fixed at dusk; a result strongly suggesting an influence exerted by light on the cell divisions and nuclear activity of the pathogen.

In spite of the simultaneous occurrence of oozing of the pycnial nectar and the presence of numerous flexuous hyphae at the mouths of the pycnia, attempts to observe copulation between the pycniospores and the flexuous hyphae were unsuccessful.

The process followed in the initiation of the 'dicaryon' in this rust is illustrated in Figs. 1–5. The seat of the process, as in most macrocyclic rusts, is the aecial primordium. The primary aecial primordia are composed of rows of uniformly uni-nucleate, irregularly polygonal cells (Fig. 1). The initiation of the dicaryon occurs at this stage between adjacent cells and is very similar to the process observed by Allen (1933), Colley (1918), and Fromme (1912) in *Puccinia coronata*, *Cronartium ribicola* and *Melampsora lini*, respectively. The process starts with partial dissolution of the wall between the two fusing cells, which lie side by side in the same row (Fig. 2). No fusion has been observed between cells located in

different rows. The fusing cells, eventually, elongate to two to four times their original size, the intervening wall completely disappears, and the two nuclei approach each other and lie as a conjugate pair. Ultimately



Figs. 1–5. *Puccinia penniseti*. Fig. 1. Primary aecial primordium showing uninucleate cells; × 525. Fig. 2. The same, later state showing cell fusions (*A*); × 370. Figs. 3, 4. Cell fusions; × 1525. Fig. 5. Two-legged cell with chain of binucleate aecidiospores. × 850.

2-legged cells (Figs. 3, 4), typical of *Cronartium ribicola* as figured by Colley (1918), develop. The fusing cells and the two nuclei forming the dicaryon are not differentiated either in size or shape.

The further development of the secondary aecial primordium and consequent formation of the chains of binucleate aeciospores proceed as shown in Fig. 5.

On the basis of these observations, it may be concluded that dicaryotization in *Puccinia penniseti* Zim. follows a pattern closely similar to that of *Cronartium ribicola* and *Melampsora lini* (Colley, 1918; Fromme, 1912). No nuclear migrations were observed in the aecial primordia of this rust at any stage, suggesting that the manner of initiation of the dicaryon in this rust is constant, and is of the 'Christman Type' (cell fusion). It may be of interest in this connexion to note that in macrocyclic rusts investigated so far, the mode of initiation of the dicaryon is of either types but never both, although both types have been recorded by Lamb (1934) in a microcyclic rust.

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REFERENCES

Allen, R. F. (1933). A cytological study of heterothallism in *Puccinia coronata*. *J. agric. Res.* **45**, 513–541.

Blackman, V. H. (1904). On the fertilization, alternation of generations and general cytology of the Uredineae. *Ann. Bot., Lond.*, **18**, 323–373.

Christman, A. H. (1905). Sexual reproduction in the rusts. *Bot. Gaz.* **39**, 267–275.

Colley, R. H. (1918). Parasitism, morphology and cytology of *Cronartium ribicola*. *J. agric. Res.* **15**, 619–659.

Fromme, F. D. (1912). Sexual fusion and spore development of the flax rust. *Bull. Torrey bot. Cl.* **39**, 113–131.

Lamb, I. M. (1934). On the morphology and cytology of *Puccinia prostii*, a microform with pycnidia. *Trans. roy. Soc. Edinb.* **58**, 143–162.

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RESISTANCE MECHANISMS OF SPECIES OF *TRITICUM* AND *AEGILOPS* AND OF AMPHIDIPLOIDS BETWEEN THEM TO *ERYSIPHE GRAMINIS* DC.

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(With Plate 2 and 10 Text-figures)

Seedling leaves of a number of species of *Triticum* and *Aegilops* were inoculated with conidia of a single race of *Erysiphe graminis tritici*. In resistant species there were at least three stages at which development of the parasite could be halted, depending partly on the conditions under which the plants were growing and partly on the species inoculated. The three stages each involve different mechanisms of resistance, which are discussed in relation to the problem of the physical or chemical nature of host-parasite relationships in obligate parasites.

Resistance to powdery mildew, *Erysiphe graminis* DC., is shown by several species of *Triticum* and *Aegilops*. Mains (1933) found that most varieties of the commonly cultivated wheats (*Triticum compactum*, *T. spelta*, *T. vulgare*, *T. durum* and *T. turgidum*) were susceptible to this disease, but that the species *T. monococcum*, *T. carthlicum*, *T. dicoccoides*, *T. dicoccum* and *T. timopheevi* were resistant although some susceptible lines were found. Bell & Lupton (1955), using a single race of mildew, found sources of resistance in *T. carthlicum*, *T. dicoccum*, *T. monococcum*, *T. timopheevi* and in numerous diploid and tetraploid species of *Aegilops* (*A. caudata*, *A. longissima*, *A. sharonensis*, *A. speltoides*, *A. biuncialis*, *A. cylindrica*, *A. ovata* and *A. triuncialis*). Bell & Lupton (1955) also investigated the mildew susceptibility of amphidiploids formed by colchicine treatment of sterile hybrids between mildew resistant and mildew susceptible species. The mildew reactions of these amphidiploids suggested that several genetic factors were involved in mildew resistance, different species of *Triticum* and *Aegilops* carrying different factors for resistance.

There is no reason to suppose that all the genetic factors for resistance to mildew should operate at the same point in the infection process. Corner (1935) distinguished two forms of resistance in species of *Triticum*. In one, the germinating conidium formed an infection papilla, but haustoria and secondary germ tubes were never produced. In the other, penetration of the epidermal cell-wall occurred, but the haustoria formed were small and distorted, and only a limited amount of surface mycelium developed. These observations were confirmed by Smith & Blair (1950). Cherewick (1944) distinguished a third method of resistance in which the haustoria developed almost as well as in susceptible varieties, but the infected cells were killed so that the fungus was unable to develop. Plants showing this type of resistance could be distinguished by macroscopically distinguishable necrotic spots. White & Baker (1954), working with barley, found that the early stages of infection were the same in all the varieties they examined,

the first haustorium being formed between 30 and 48 hr. after inoculation, but that in the more resistant varieties collapse of the mesophyll cells below the point of infection began as soon as the first haustorium was formed, resulting in a halting of further fungal growth. In less resistant hosts a larger number of haustoria were developed before collapse of the mesophyll cells began, and in fully susceptible varieties unrestricted development of haustoria was possible without any collapse of mesophyll cells.

Workers with other species of *Erysiphe* have demonstrated other mechanisms of resistance. Neger (1923) showed that resistance to *E. cichoracearum* was brought about by enclosure of the haustoria of the fungus in a gummy substance which prevented further development. Mackie (1928) was, however, unable to find any trace of such a resistance mechanism in barley. Smith (1938) showed that in clover varieties resistant to *E. polygoni* the infection hyphae penetrated the cuticle and entered the epidermal cells of the host, but progressed no further. The infected host cells, however, stained deeply with safranin and died shortly after penetration. This necrotic effect was sometimes confined to a single host cell and was sometimes seen in a group of adjacent cells.

The present paper describes the early stages of infection of *Erysiphe graminis tritici* on a number of species of *Triticum* and *Aegilops* and on certain amphidiploids derived from them, and demonstrates that there are at least three stages at which development of the parasite may be checked when a resistant plant is inoculated.

Materials and methods

Seedlings of resistant and susceptible species of *Triticum* and *Aegilops* and of certain amphidiploids between them as listed below were inoculated with conidia of *Erysiphe graminis tritici*. The cross numbers in parentheses are those of Bell, Lupton & Riley (1955) and the disease reactions are those of Bell & Lupton (1955). Resistant species:

Triticum carthlicum Nevski (= *T. persicum* Vav.)
T. dicoccum Schübl. var. *farrum* Körn.
T. dicoccum Schübl. sub. sp. *georgicum* Dekap. et Menab.
T. timopheevi Zhuk. var. *typicum* Zhuk.
Aegilops caudata L. var. *typica* Eig
A. speltoides Tausch. var. *Aucheri* (Boiss.) Born.
A. ovata L. var. *vulgaris* Eig

Susceptible species:

Triticum durum Desf. var. *caerulescens* Körn.
T. turgidum L. var. *iodurum* Körn.

Resistant amphidiploids:

T. dicoccum var. *farrum* × *T. timopheevi* (TA 197)
A. caudata × *T. dicoccum* var. *farrum* (TA 72)
A. caudata × *T. timopheevi* (TA 248)
T. durum × *A. ovata* (TA 280)
T. timopheevi × *A. ovata* (TA 281)
T. dicoccum var. *farrum* × *A. ovata* (TA 286)

Susceptible amphidiploids:

T. timopheevi × *T. turgidum* (TA 211)
T. timopheevi × *T. durum* (TA 212)
T. carthlicum × *T. timopheevi* (TA 228)
A. caudata × *T. turgidum* (TA 247)
A. caudata × *T. durum* (TA 249)

Seedlings were inoculated at the two-leaf stage by shaking heavily sporulating plants above them. They were then placed under glass covers in a tray of water for 48 hr. Great difficulty was experienced in controlling the conditions so as to ensure satisfactory development of haustoria on the susceptible species which were used as controls. It was normally found, however, that between 25 and 50 % of the germinating spores produced haustoria on the susceptible species when experiments were carried out in a well-lit greenhouse at a temperature of 15–18° C. Experiments in which a satisfactory infection of the controls was not obtained were disregarded.

Leaves were fixed for examination at times ranging from 2 to 7 days after inoculation. Fixation was carried out by immersion in a mixture of 3 parts pyridine: 1 part water. Leaves were kept in this mixture at 30° C. for 4 days during which time the chlorophyll was bleached and the leaves rendered translucent. The leaves were then washed in repeated changes of water, stained in acetic cotton blue made up as described by Noll (1951) and mounted in dilute glycerine for examination. Leaves showing features of interest when examined in this way were embedded in paraffin wax and sectioned on the microtome at 10μ.

The race of mildew used for these investigations was identified as race 4 by the use of the differential hosts Axminster, Chul, Huron and Norka as described by Newton & Cherewick (1947).

Results

It was found that the behaviour of the susceptible species tested (*Triticum durum* and *T. turgidum*) differed widely under different conditions of inoculation, and that differences in behaviour were frequently seen in different parts of the same leaf. Under conditions favourable for infection, the conidia produced germ tubes and appressoria which penetrated the cuticle and the epidermal cell-walls, large finger-shaped haustoria and abundant surface mycelium being developed, as described by Corner (1935) (Pl. 2, figs. 1, 2; Text-figs. 1, 2). Under other conditions, although normal germ tubes and appressoria were produced, the epidermal cell-walls were not penetrated and characteristic blue staining 'haloes' were found surrounding the points at which the germ tubes had passed through the cuticle. This behaviour, which was also shown by certain of the mildew resistant species, is discussed below. It was found on certain leaves that a proportion of the appressoria were associated with these 'haloes' and a proportion with successful penetration accompanied by the development of haustoria and surface mycelium. Occasionally 'haloes' were associated with successful penetrations.

The resistant species examined demonstrated three distinct forms of resistance mechanism. When leaves of *T. carthlicum*, *T. dicoccum* var. *farrum* or sub.sp. *georgicum*, *T. timopheevi* or *Aegilops caudata* were inoculated it was found that although germ tubes and appressoria were produced, the fungus was unable to penetrate the epidermal cell-walls. The behaviour seen was similar to that found when a susceptible species was inoculated under conditions unfavourable for infection. The cuticle was penetrated as in a susceptible host, and in cotton blue stained preparations the point of penetration was surrounded by a blue stained 'halo', the size of which varied according to the species inoculated. The 'haloes' were largest in the two forms of *Triticum dicoccum* and were progressively smaller in *T. carthlicum*, *T. timopheevi* and *Aegilops caudata* (Pl. 2, figs. 3–5; Text-figs. 3–5).

In transverse section, it was found that the epidermal cell walls were much thickened below the points at which the cuticle had been penetrated. The thickened portion was very deeply stained in these resistant varieties, though in susceptible controls little staining took place, and it was possible to trace the path of the fungus through the cuticle and the epidermal cell-wall to the haustorium below (Pl. 2, fig. 2; Text-fig. 2).

When *A. ovata* was inoculated it was found that penetration normally took place as in susceptible species, but that the haustoria formed were small. A limited amount of surface mycelium was developed for the first 3 or 4 days after infection, but after this time the haustoria and mycelium degenerated and died (Pl. 2, figs. 6, 7; Text-figs, 6, 7). As with susceptible species inoculated under conditions unfavourable for infection, it was sometimes found that the fungus failed to penetrate the epidermal cell-wall. Under these circumstances the cell-wall became very much swollen around the point at which the cuticle had been penetrated, the swollen portion staining very deeply with cotton blue (Pl. 2, fig. 8; Text-fig. 8).

A third mechanism of resistance, corresponding to that observed by Smith (1938) in clover inoculated with *Erysiphe polygoni*, was seen when leaves of *Aegilops speltoides* were inoculated. The cuticle and epidermal cell-walls were penetrated as in susceptible species, but no haustoria were developed since the penetrating hypha burst as soon as the cell-wall had been penetrated by the fungus, killing the cell contents. The dead cells stained deeply with cotton blue; the stain was sometimes confined to the cell which the fungus had penetrated, but at other times two or three of the surrounding epidermal cells were also stained (Pl. 2, fig. 9; Text-fig. 9).

No evidence was obtained to suggest that spore germination was influenced by the host. Normal spore germination was even seen on a leaf of *Vicia faba*. Appressoria were produced in this case, but no 'haloes' were visible when the leaf was stained with cotton blue, suggesting that the cuticle had not been penetrated (Pl. 2, fig. 10; Text-fig. 10). In none of the species inoculated was there any suggestion of the formation of a gummy substance within the host preventing the development of the fungus after the formation of the first haustorium, as found by Neger (1923) with *Erysiphe cichoracearum*.

Four interspecific amphidiploids involving *Triticum timopheevi* were examined. Three of these (*T. carthlicum* × *T. timopheevi*, *T. timopheevi* ×

T. durum and *T. timopheevi* × *T. turgidum*) were susceptible to mildew as seedlings (Bell & Lupton, 1955) though they showed no infection as mature plants in the field. Each showed the development of haustoria and mycelium when cleared and stained. It was noted, however, that in the cross involving *T. carthlicum* the haustoria were small and somewhat distorted. The behaviour of this cross is of particular interest, since although itself susceptible, both its parents were resistant to mildew. The fourth interspecific amphidiploid (*T. dicoccum* var. *farrum* × *T. timopheevi*) was resistant as a seedling. On clearing and staining it was found that although most of the appressoria failed to cause infection but were associated with 'haloes', a very small proportion produced normal infection with the development of haustoria and a limited amount of surface mycelium. This type of successful infection was also occasionally seen in *T. timopheevi*.

Four intergeneric amphidiploids involving *Aegilops caudata* were examined. Two of these (*A. caudata* × *Triticum turgidum* and *A. caudata* × *T. durum*) were susceptible as seedlings, though resistant as mature plants in the field. They showed abundant haustoria and surface mycelium in the cleared preparations. The other two crosses (*A. caudata* × *T. dicoccum* var. *farrum* and *A. caudata* × *T. timopheevi*) were resistant both as seedlings and as mature plants. In cleared preparations most of the appressoria on these crosses were associated with 'haloes', but in a small proportion successful infection had taken place with the development of haustoria and a limited amount of surface mycelium.

In the three amphidiploids involving *Aegilops ovata* (*Triticum dicoccum* var. *farrum* × *A. ovata*, *T. durum* × *A. ovata* and *T. timopheevi* × *A. ovata*), all of which were resistant to mildew as seedlings, degenerate haustoria and a limited amount of surface mycelium were produced.

Discussion

The differences found in the resistance mechanisms of species and amphidiploids to *Erysiphe graminis* recall the suggestion of Sempio (1939) that protoplasmic resistance to fungi in higher plants may take either of the two forms: (i) toxological resistance—resulting from the presence of toxins elaborated or absorbed by the plant; or (ii) metabolic resistance—due to an acceleration or retardation of metabolism, resulting in soluble components of protoplasm becoming less attractive to the parasite, which is consequently starved.

The nature of resistance to mildew was discussed very fully by Smith & Blair (1950), who recalled Chester's view on obligate parasitism, made with regard to the leaf rusts, but no less applicable here. Chester (1946) pointed out that when the range of higher plants is considered, the number of hosts which were susceptible to attack by any given obligate parasite were in a very small minority. He put the problem of susceptibility and resistance in a more positive light, suggesting that the susceptible host must possess some chemical which is necessary for infection rather than be lacking in some deleterious substance. This chemical he suggests is more likely to be a protein than a carbohydrate or inorganic compound. Smith & Blair (1950) suggested that the protein concerned was either a food

base used by the fungus or a protein anti-toxin counteracting toxins produced by the fungus.

It appears that for *E. graminis* there are at least four stages at which invasion of the host may be halted:

(*a*) The fungus may form appressoria but be incapable of penetrating the cuticle.

(*b*) The fungus may form appressoria capable of causing penetration of the cuticle, but not capable of penetrating the epidermal cell walls, which become much thickened below the point at which the cuticle is penetrated.

(*c*) The cuticle and epidermal cell walls may be penetrated, but the invasion of the host be halted at this stage owing to the hypersensitivity of the epidermal cells which are killed on contact with the fungus.

(*d*) Haustoria may be formed in the epidermal cells but these may degenerate and die within a few days of their formation.

When the invasion of the host is halted before the cuticle has been penetrated, as in (*a*), the host may be considered immune to the fungus, as no response on the part of the host is required to throw off the fungal attack. This type of behaviour is only seen, however, when a completely unsuitable host, such as *Vicia faba*, is inoculated. In the other types a greater or lesser response on the part of the host is necessary before the advance of the fungus is halted, so that the host should be considered resistant but not immune.

As has been noted, resistance of type (*b*), normally exhibited by *Triticum carthlicum*, *T. dicoccum*, *T. timopheevi* or *Aegilops caudata* is also seen when susceptible wheats are inoculated under conditions unfavourable for infection. It therefore seems likely that resistance of this sort is controlled by some minor physiological difference between the cells of susceptible and resistant hosts. This difference must be a relatively small one since resistant and susceptible reactions are sometimes seen on different portions of the same leaf and even in one microscope field. Cell turgor is suggested as a factor which may possibly be involved. This physiological change may perhaps determine the availability of the food base or protein anti-toxin suggested by Smith & Blair (1950). Alternatively, the effect may be entirely physical, altering the capacity of the cell-wall to resist penetration by the fungus. Evidence of the physical nature of this effect has been obtained by Dickinson (personal communication).

The appearance of a 'halo' on staining with cotton blue indicates some change in the nature of the cell-wall surrounding the point at which the cuticle has been penetrated. Further evidence of this is provided by the swelling seen in the epidermal cell-walls in this zone, and suggests that 'halo' production is the manifestation of a 'bruising' of the epidermis below the point of penetration. This 'bruising' may be caused by the diffusion of an enzyme produced by either host or parasite at the point of penetration, or may be a purely mechanical effect of the physical action of the fungus as it attempts to penetrate the epidermal cell wall. When a susceptible host is inoculated under conditions favourable for infection, the epidermal cell walls are penetrated with little difficulty, and are not 'bruised', so that no 'halo' is seen around the point of penetration, though Pl. 1, fig. 2, shows that even here there is some disturbance of the epider-

mal cell-walls, fragments of which can be seen around the penetrating hypha. When conditions are unfavourable for infection, a much greater distortion of the cell-walls is caused as the fungus attempts but fails to penetrate, resulting in a strain being transmitted to the surrounding areas of cell-wall, which are damaged in such a way as to swell up and to take up the stain if mounted in cotton blue. A similar distortion is caused when *Triticum carthlicum*, *T. dicoccum*, *T. timopheevi* or *Aegilops caudata* are inoculated, the different sizes of the 'haloes' produced when these species are inoculated depending on the sizes of the 'bruises' formed. Further evidence in support of this theory is provided by the appearance of 'haloes' associated with successful penetrations sometimes seen when susceptible species are inoculated. In such cases it seems likely that penetration has been slow, and has been associated with a considerable distortion and bruising of the epidermal cell-walls before they were penetrated by the fungus.

Resistance of type (*c*), shown by *A. speltoides*, appears to correspond to the toxological resistance suggested by Sempio (1939), as the death of the epidermal cells of the host is apparently due to a toxic interaction between the cell contents and the fungus. It is reminiscent of Dickinson's observation of the bursting of appressoria when spores of *Erysiphe graminis* are germinated on certain artificial membranes (Dickinson, 1949).

The mechanism of resistance shown by *Aegilops ovata* (type *d*) seems to correspond well to the metabolic resistance of Sempio. This form of resistance would also, when less pronounced, account for the intermediate stages between complete immunity and full susceptibility seen in other species and in some of the amphidiploids. This type of resistance and that shown by *A. speltoides* can, however, only be called into action after the host cell has been penetrated. The evidence, therefore, suggests that resistance to the penetration of the cuticle and of the epidermal cell-walls is physical in nature, and that only after these barriers have been breached are others of a chemical or nutritional nature called into play to prevent or limit further development of the fungus.

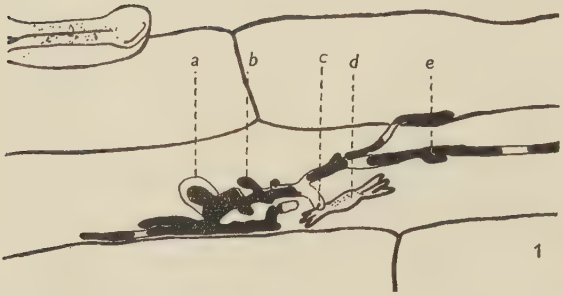
The author acknowledges with gratitude the help and advice of Dr S. Dickinson of the School of Agriculture, Cambridge. He also wishes to acknowledge the assistance of Mr H. P. Hughes of the Cambridge Plant Breeding Institute, who has done much of the routine work and has prepared all the photographs and text-figures.

The amphidiploids used in this investigation form part of a series of interspecific and intergeneric amphidiploids in the Triticinae developed at the Cambridge Plant Breeding Institute. The author wishes to acknowledge the help received in making this material available for his use.

REFERENCES

Bell, G. D. H. & Lupton, F. G. H. (1955). Investigations in the Triticinae. IV. Disease reactions of species of *Triticum* and *Aegilops* and of amphidiploids between them. *J. agric. Sci.* **46**, 232–246.

Bell, G. D. H., Lupton, M. & Riley, R. (1955). Investigations in the Triticinae. III. The morphology and field behaviour of the A_2 generation of interspecific and intergeneric amphidiploids. *J. Agric. Sci.* **46**, 199–231.



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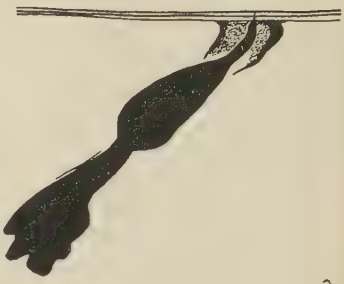

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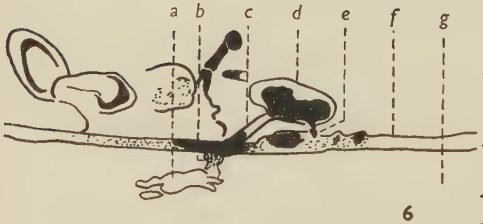

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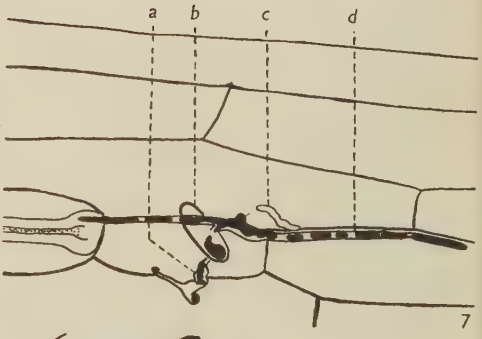

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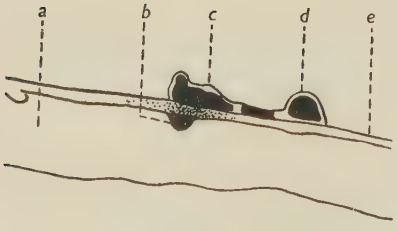

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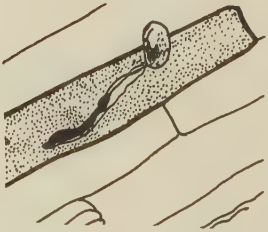

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Text-figs. 1–10. For explanation see p. 59.

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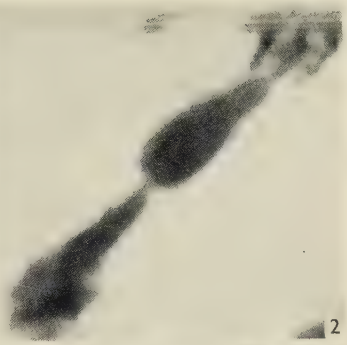
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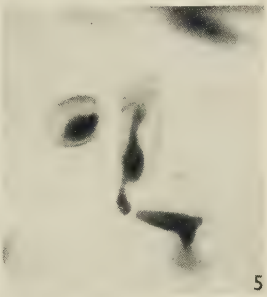
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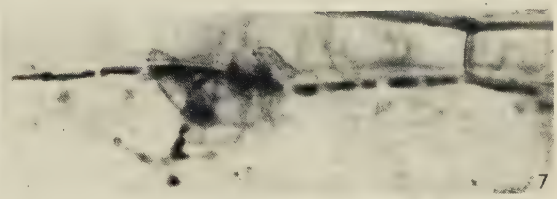
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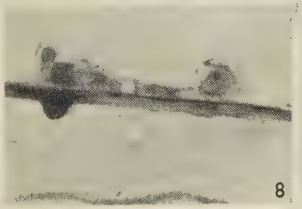
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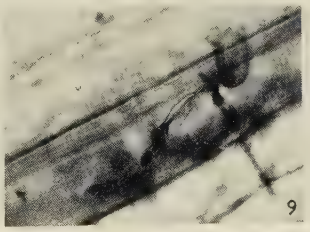
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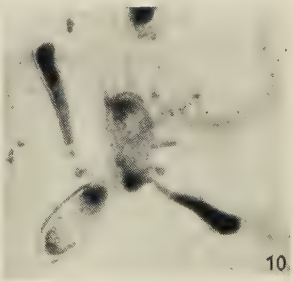
10

(*Facing p.* 58)

Cherewick, W. J. (1944). Studies on the biology of *Erysiphe graminis* DC. *Canad. J. Res.*, C, **22**, 52–86.
Chester, K. S. (1946). *The cereal rusts*. Waltham, Mass.: Chronica Botanica Co.
Corner, E. J. H. (1935). Observations on resistance to powdery mildews. *New Phytol.* **34**, 180–200.
Dickinson, S. (1949). Studies in the physiology of obligate parasitism. (4) The formation on membranes of haustoria by rust hyphae and powdery mildew germ tubes. *Ann. Bot., Lond.*, **13**, 345–353.
Mackie, J. R. (1928). Localization of resistance to powdery mildew in the barley plant. *Phytopathology*, **18**, 901–910.
Mains, E. B. (1933). Host specialization in *Erysiphe graminis tritici*. *Proc. Nat. Acad. Sci. Wash.* **19**, 49–53.
Neger, F. W. (1923). Beiträge zur Biologie der Erysipheen. iii Der Parasitismus der Mehltaupilze—eine Art von geduldeter Symbiose. *Flora*, **16**, 331–335.
Newton, M. & Cherewick, W. J. (1947). *Erysiphe graminis* in Canada. *Canad. J. Res.*, C, **25**, 73–93.
Noll, A. (1951). Über mikroskopische Anfangssymptome der Resistenz und Anfälligkeit von Weizensorten gegen *Puccinia glumarum*. *Phytopath. Zeit.* **17**, 400–405.
Sempio, C. (1939). Aspetti del Problema della resistenza in patologia vegetale. *Atti e Communicazioni. IV. Congr. int. Pat. comp., Roma*, 1939. Pp. 355–366.
Smith, H. C. & Blair, I. D. (1950). Wheat powdery mildew investigations. *Ann. appl. Biol.* **37**, 570–583.
Smith, O. F. (1938). Host-parasite relations in red clover plants resistant and susceptible to powdery mildew—*Erysiphe polygoni*. *J. agric. Res.* **57**, 671–682.
White, N. H. & Baker, E. P. (1954). Host relations in powdery mildew of barley. I. Histology of tissue reactions. *Phytopathology*, **44**, 657–662.

EXPLANATION OF PLATE 2 AND TEXT-FIGURES 1–10

Fig. 1 (Text-fig. 1). Surface view of *Triticum durum* 3 days after inoculation with *Erysiphe graminis* showing typical behaviour of a susceptible variety. *a*, conidium; *b* and *e*, secondary appressoria; *c*, primary appressorium; *d*, typical haustorium.

Fig. 2 (Text-fig. 2). Transverse section of *Triticum vulgare* 3 days after infection with *Erysiphe graminis* showing path of fungus through the cuticle and thickened epidermal cell wall to form haustorium in epidermal cell.

Fig. 3 (Text-fig. 3). Surface view of *Triticum dicoccum* 3 days after inoculation with *Erysiph graminis* showing large dark staining 'halo' surrounding appressorium of the fungus.

Fig. 4 (Text-fig. 4). Surface view of *Triticum carthlicum* 3 days after inoculation with *Erysiphe graminis* showing typical 'haloes' surrounding appressoria of the fungus.

Fig. 5 (Text-fig. 5). Surface view of *Triticum timopheevi* 3 days after inoculation with *Erysiphe graminis* showing typical 'halo' surrounding appressorium of the fungus.

Fig. 6 (Text-fig. 6). Transverse section of *Aegilops ovata* 7 days after inoculation with *Erysiphe graminis*. *a*, degenerate haustorium; *b*, swelling in epidermal cell-wall below appressorium; *c*, primary germ tube; *d*, conidium; *e*, tertiary germ tube; *f*, cuticle of host; *g*, cavity of epidermal cell.

Fig. 7 (Text-fig. 7). Surface view of *Aegilops ovata* 7 days after inoculation with *Erysiphe graminis*. *a*, tertiary germ tube; *b*, conidium; *c*, degenerate haustorium; *d*, degenerate mycelium.

Fig. 8 (Text-fig. 8). Transverse section of *Aegilops ovata* 7 days after inoculation with *Erysiphe graminis*. *a*, cavity of epidermal cell; *b*, deeply stained swelling of epidermal cell-wall associated with unsuccessful penetration; *c*, primary germ tube and appressorium; *d*, conidium; *e*, cuticle of host.

Fig. 9 (Text-fig. 9). Surface view of *Aegilops speltoides* 3 days after inoculation with *Erysiphe graminis* showing appressorium of fungus over deep staining dead epidermal cell.

Fig. 10 (Text-fig. 10). Surface view of *Vicia faba* 3 days after inoculation with *Erysiphe graminis*, showing development of germ tubes but absence of 'haloes'.

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NOTES ON *TAPHRINA*

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(With 16 Text-figures)

The symptoms caused by *Taphrina tosquinetii* (Westend.) Magnus on the shoots and leaves of alder (*Alnus glutinosa*) are fully described, and an account given of its morphology and cultural characteristics. The correct author citation for the species is discussed. A description is given of *T. ulmi* (Fkl.) Johans. affecting the leaves of elms (*Ulmus* spp.) in Britain, where it is believed to be more common than has been realized hitherto. *T. minor* Sadeb., commonly associated with leaf curl of cherries (*Prunus avium*) and related species, is accepted as identical with *T. cerasi* (Fkl.) Sadeb. causing witches' brooms on the same hosts; and an occurrence on the ornamental species *Prunus subhirtella* is noted.

(1) **Taphrina tosquinetii** (Westendorp) P. Magnus

Symptoms (Figs. 1–3). This is by far the commonest of the three species of *Taphrina* on *Alnus glutinosa*, and usually affects every tree in a given locality. The most characteristic symptom is the systemic distortion of the water-shoots (suckers) which arise principally from the base of the trunk but have been observed to spring from within the crown at about 20 ft. from the ground. Usually, all the suckers on a tree are diseased; in exposed situations the side of the tree facing the prevailing wind may remain relatively free from infection, perhaps because the diseased shoots under these conditions tend to defoliate early, before the leaves become asciferous. Affected suckers frequently die back in their first or second year as noted by Henderson (1954), but may persist 10–12 years, although with increasing loss of apical dominance. There is, however, no tendency to the formation of typical witches' brooms. The diseased shoots have a thick and wrinkled cortex so that when young they may appear almost fasciated; they come into leaf a few days later on the average than the healthy shoots, and are further distinguished by the reddish colour and crumpled appearance of the young unfolding leaves. Later, the diseased leaves are easily recognized by their size and rugosity and by the bloom of the developing asci on one or both surfaces. Once affected in this way, the shoots have not been observed to recover; although, very exceptionally, an odd healthy lateral may develop from an otherwise diseased shoot system. On the other hand, it is not uncommon for the basal leaves of a newly affected shoot to remain healthy, indicating the relatively late onset of the infection. In addition to the systemic form of the disease affecting the shoots, there is also a localized infection of the leaves. Occasionally, virtually the whole leaf lamina is affected, when care is needed to distinguish this condition from that previously described; but the more characteristic symptom is

the production of one or more blisters or bullate deformations, sharply distinguished from the adjacent healthy tissues. Such partially affected leaves are found mainly in the middle region of the long shoots, or at the base of the smaller spurs, which suggests that this phase of the disease is

Figs. 1–3. *Taphrina tosquinetii.* Symptoms on *Alnus glutinosa.*

Fig. 1. Typical 2nd- or 3rd-year systemic infection of a sucker shoot.

Fig. 2. Systemic infection of 1st-year lateral; the terminal growth and the other laterals unaffected.

Fig. 3. Varying degrees of localized infection of individual leaves.

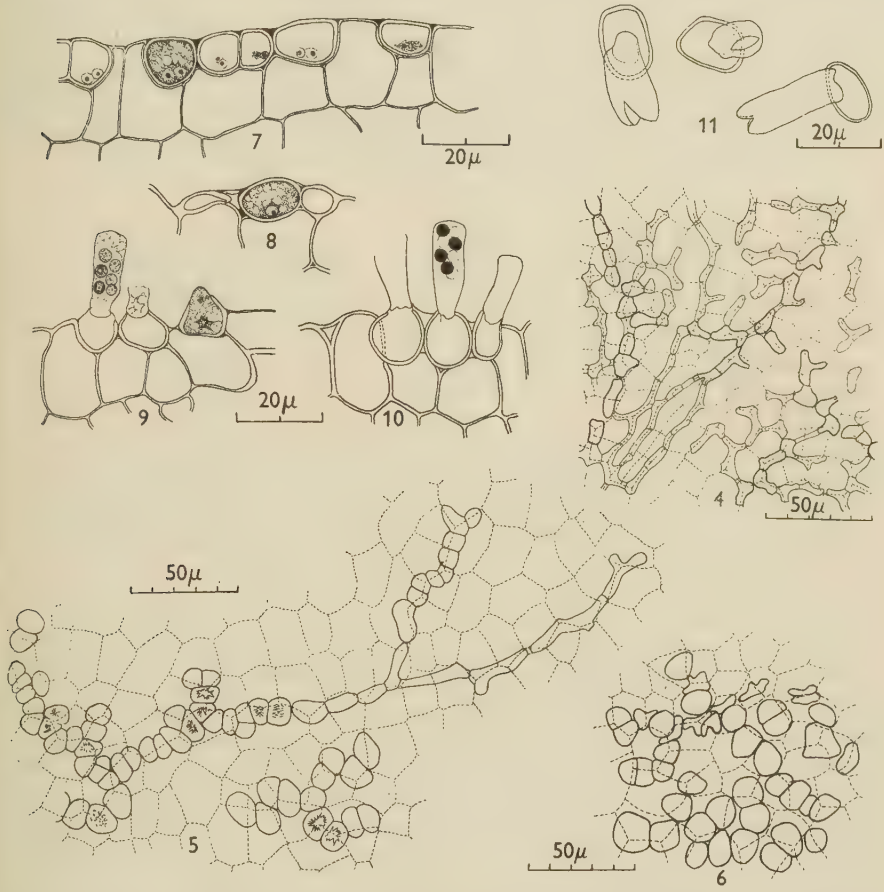
secondary to the systemic infection of the shoots. It is not likely to be confused, in the field, with the distinctive yellow leaf-spotting caused by *Taphrina sadebeckii* Johanson (Henderson, 1954). Observations on a mixed plantation of Common Alder (*Alnus glutinosa*) and Grey Alder (*A. incana*) in the vicinity of Sheffield in 1948–9 showed that *Taphrina tosquinetii* was confined to the former (on which, as usual, it occurred abundantly).

T. amentorum (Sadeb.) Rostrup, reported from both species of alder as infecting the female catkins, was not found in this locality; nor was *T. sadebeckii*.

Morphology (Figs. 4–11). The mycelium, on both surfaces of the leaf, is entirely subcuticular. Surface mounts from the young, unfolding, leaves show that it consists of narrow, thin-walled, septate hyphae passing above the radial walls of the epidermal cells. The appearance at this stage suggests an initial phase of rapid extension growth, followed by copious branching, so that eventually the hyphae form a more or less continuous network. The hyphal elements then become resolved into chlamydospores—the ascogenous cells—which themselves appear capable of multiplying further by budding, in the manner figured by Henderson (1954). Thus, by the time the leaf is fully expanded, the ascogenous cells may be more or less contiguous, completely covering the epidermis. In surface view they are rounded to somewhat flattened by mutual pressure, of variable size, averaging about $15 \times 11 \mu$. They are shallowly inserted between the epidermal cells, about 12μ in depth. During their formation, the ascogenous cells (and the cells of the hyphae from which they are derived) are binucleate. Nuclear fusion occurs at their maturity, and is immediately followed by the extrusion of the cell contents into a thin walled papilla representing the young ascus. When fully developed, this is cut off by a cross-wall, leaving the basal cell, as it is now called, apparently empty. The ripe asci are cylindrical or slightly enlarged above, subtruncate at the apex, rather uniform in size, about $28 \times 9 \mu$. Typically, they contain eight spherical, uni-nucleate, ascospores which appear to be bi-seriate at first, or to be formed in two super-imposed groups of four. The details of nuclear behaviour have not been fully worked out; though preliminary observations suggest that the successive divisions occur, almost simultaneously, towards the tip of the ascus as described by Yarwood (1941) for *T. deformans*. The ascospores are either discharged immediately, through a wide apical slit (the ascus dehiscence is bilabiate), or they remain within the ascus and commence to multiply by budding. Thus the proportions of octosporous and of polysporous asci will be found to vary according to the age and antecedent conditions for the material examined. Only the octosporous asci are capable of active discharge: the contents of the polysporous asci are liberated passively.

Cultural characters (Figs. 12–14). Cultures were invariably obtained by upward discharge of the ascospores on to thin films of malt agar or other similar media. The ascospores, within a few hours of discharge, form secondary spores by a process of budding, building up typical yeast-like colonies which increase slowly in diameter to an observed maximum of about 2 cm. The colour of the colonies is at first whitish, then pale pink. In the older colonies, certain enlarged, thick-walled, cells occur which may possibly be homologous with the ascogenous cells (cf. Mix, 1935); and, very occasionally, some of the ascospores produce short germ tubes. Otherwise, the colonies consist entirely of variously shaped, thin-walled, blastospores similar in appearance to those commonly found on the surface of infected leaves, and within the polysporous asci. The colonies were found to resist desiccation for periods of more than a year at room temperature.

Nomenclature. There has been much unnecessary confusion between the three *Taphrina* spp. on alder (*Alnus glutinosa*) in Britain. Henderson (1954) now clearly distinguishes them; but both he and Mix (1949) are in error in attributing the valid name for the present species, *Taphrina tosquinetii*, to Tulasne (1866). The facts are as follows. Westendorp (1861) first described the fungus, causing bullate deformations of the leaves of *Alnus glutinosa*, in



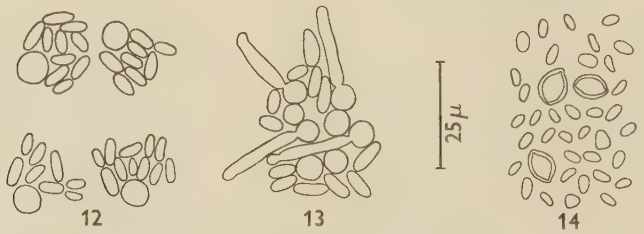
Figs. 4–11. *Taphrina tosquinetii*. Morphology in host.

Figs. 4–6. Surface preparations viewed through the upper epidermis. In Fig. 4 the (subcuticular) mycelium is still largely hyphal in nature, and the young ascogenous cells have not yet ruptured the cuticle. Fig. 5 illustrates an unusually extensive and isolated hyphal branch, with the formation of ascogenous cells proceeding acropetally. Rupture of the cuticle is just commencing. In Fig. 6 the majority of the cells bear mature eight-spored asci (not shown). The irregularly shaped cells in the upper part of the figure belong to asci which have already discharged their spores.

Figs. 7–10. Transverse sections at 5μ. Figs. 7–8 correspond approximately to the stage of Figs. 4–5: note the binucleate condition of the hyphal cells and, in Fig. 8, the single fusion nucleus. Fig. 9 shows (right) the first emergence of the ascus and (left) a mature ascus, with uninucleate ascospores. In Fig. 10, a group of mature and discharged asci, note the chlamydospore-like appearance of the stalk cells.

Fig. 11. Discharged asci *in situ* (from a surface preparation), showing bilabiate dehiscence.

Belgium, as *Ascomyces tosquinetii* sp.nov. The material had been communicated to him by M. Tosquinet, previously (1852) identified as '...notre collègue et ami..., médecin de bataillon au 2e régiment de ligne à Charleroi'. Tulasne (1866) effected the transfer to *Taphrina* Fr. in his monograph of that genus, using the (illegitimate) combination *T. alnitorqua* Tul., and quoting *Ascomyces tosquinetii* Westend. as a synonym. He describes fully the systemic distortion of the shoots, noting also that the leaves may be affected only in part. Both forms of the disease were first recorded for Britain, under *A. tosquinetii* West. (with *Taphrina alnitorqua* Tul. as a synonym), by Phillips & Plowright (1877). Finally, Magnus (1890), after examining Westendorp's type material in Brussels, confirmed that it was covered by Tulasne's description and formally proposed the combination *T. tosquinetii* (West.) P. Magnus, which is now accepted as correct.



Figs. 12–14. *Taphrina tosquinetii*. Morphology in culture.

Fig. 12. Four of the eight ascospores from a single ascus, about 21 hr. after discharge on to agar, separated by micro-manipulator.

Fig. 13. A colony about 26 hr. after discharge, in which, exceptionally, four of the ascospores have produced short germ tubes.

Fig. 14. Cell types from a 16-week colony on potato-dextrose agar. Note the occasional thick-walled (empty) cells, possibly homologous with the ascogenous cells.

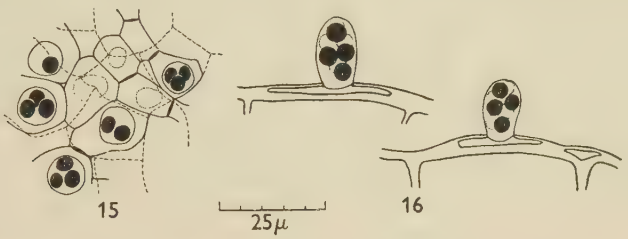
(2) **Taphrina ulmi** (Fuckel) Johanson

This species is not listed for Britain by Ramsbottom & Balfour-Browne (1951); but it was recorded by Mix (1949), from Kent. According to Henderson (1954), it is not found in Scotland. It is now recorded as common on hedgerow elms (mainly *Ulmus glabra* Huds.) in north Somerset (V.C. 6) and seems likely to have been overlooked elsewhere on account of the relative inconspicuousness of the symptoms that it produces (cf. Sadebeck, 1884).

Symptoms. The disease appears to be confined to the suckers and adventitious shoots, on which it causes a diffuse blotching of the leaves. The blotches range in size from ill-defined spots a few mm. in diameter to extensive areas covering more than half the leaf. All parts of the leaf may be affected, but the possibility of primary infection via the petiole cannot be overlooked. The blotches are at first paler than the surrounding tissues, or very slightly yellowed. They become somewhat wrinkled on the upper surface (there is no other evidence of hypertrophy or distortion), and develop a scarcely discernible asciferous 'bloom'. They then rapidly dry out in the centre, commencing on the lower surface where they become distinguishable as brown necrotic patches with a broad irregular margin

of yellow. Eventually, the necrotic areas fall out, so that the leaf becomes ragged and perforated as if severely attacked by caterpillars.

Morphology (Figs. 15, 16). The mycelium is subcuticular. The asci, on both surfaces of the leaf, are relatively scanty, not forming a continuous layer visible to the naked eye as a distinct 'bloom'. According to Sadebeck (1884), this is due to the fact that only some of the cells become asciferous, the adjoining ones becoming emptied in the process. This could not be confirmed: from the material examined, it seemed more probable that the cells mature successively, and that the empty cells have in fact already produced their asci, from which the spores have been discharged. Whether ascogenous or not, the cells are entirely confined to beneath the cuticle: they are not in the least inserted between the epidermal cells and are much flattened, almost obliterated, at maturity. Consequently, they



Figs. 15–16. *Taphrina ulmi*. Morphology in host.

Fig. 15. Surface view through the upper epidermis. The ascogenous cells, though practically contiguous, have matured successively. The three in the centre bear discharged asci; another four bear mature asci each with eight ascospores; the one at the top of the figure is apparently still immature. Note the slightly thickened cross-walls.

Fig. 16. Views of asci in transverse sections, showing virtual obliteration of the 'stalk cells'.

are difficult to see in sections of the leaf. As seen in surface preparations, they are occasionally more or less contiguous on all sides, more often comprising an irregular network. They are irregularly polygonal in shape, up to 20μ or more in their longest dimension, very thin-walled except for the short transverse septa which recall in some degree the 'plate septa' of *Taphrina deformans* as figured by Mix (1935). At no stage are they at all chlamydospore-like, as in *T. tosquinetii* and many other species. The asci themselves are among the smallest in the genus, averaging about 15 × 9μ. They are ellipsoid, rounded at the apex, and contain eight spherical ascospores, which frequently bud within the ascus. Upward discharge of the ascospores on to agar media results in the production of typically yeast-like colonies. Subsequent growth in culture has been the slowest of any of the species investigated under comparable conditions, and the colour of the colonies the palest.

(3) **Taphrina cerasi** (Fuckel) Sadebeck

Syn. *T. minor* Sadebeck

Mix (1949) discusses in detail the taxonomy of the species commonly associated respectively with witches' brooms (*T. cerasi*) and leaf curl (*T. minor*) of Wild Cherry (*Prunus avium*) and related host plants, and

concludes that they are identical. Henderson (1954) accepts the synonymy but makes no explicit reference to leaf curl as such. The typical witches' brooms are well known and need no further description except to point out that the associated foliage symptoms, distortion, prolonged reddening, and eventual production of an asciferous 'bloom', are indistinguishable from those of leaf curl. Where, as frequently occurs, the two conditions are found in close proximity, it might reasonably be inferred that the leaf curl has arisen as a secondary infection from the witches' broom. Leaf curl, however, is often severe in localities from which well-developed witches' brooms are entirely absent. Detailed examination of affected trees shows that the disease tends to be confined to parts of the tree, or to individual branch systems, in which it reappears for several years in succession as if itself the result of a systemic infection. Furthermore, the branches concerned may eventually become stunted and proliferate as if affected by an incipient tendency to form witches' brooms. Thus it appears that the difference between the two diseases may be one of degree only, depending on the conditions at the moment of infection, or on other factors controlling the extent and distribution of the fungus within the tissues. The host relationships would obviously repay further investigation.

An additional host record. Typical leaf curl with, apparently, incipient witches' brooms was recorded on *Prunus subhirtella* Miquel at Westonbirt Arboretum, Glos., on 13 May 1953. It was noted again, on the same tree, a year later.

I am indebted to the Director of the Royal Botanic Gardens, Kew, for the loan of herbarium specimens, and for library facilities.

REFERENCES

Henderson, D. M. (1954). The genus *Taphrina* in Scotland. *Notes R. bot. Gdn Edinb.* **21**, 165–180.

Magnus, P. (1890). Bemerkung über die Benennung zwei auf *Alnus* lebender *Taphrina*—Arten. *Hedwigia*, **29**, 23–25.

Mix, A. J. (1935). The life history of *Taphrina deformans*. *Phytopathology*, **25**, 41–66.

Mix, A. J. (1949). A monograph of the genus *Taphrina*. *Kans. Univ. Sci. Bull.* **33**, 3–167.

Phillips, W. & Plowright, C. B. (1877). New and rare British fungi. *Grevillea*, **6**, 22–29.

Ramsbottom, J. & Balfour-Browne, F. L. (1951). List of Discomycetes recorded from the British Isles. *Trans. Brit. mycol. Soc.* **34**, 38–137.

Sadebeck, R. (1884). Untersuchungen über die Pilzgattung *Exoascus* und die durch dieselbe um Hamburg hervorgerufenen Baumkrankheiten. *Jb. Hamburg. wiss. Anst.* **1**, 91–124.

Tulasne, L. R. (1866). Super Friesiano *Taphrinarum* genere et *Acalyptospora* Mazeriana, accedente *Ustilagnis marinae* Dur. adumbratione. *Ann. Sci. nat.* (5*me sér. Bot.*), **5**, 122–136.

Westendorp, G. D. (1852). Nouvelle notice sur quelques Cryptogames récemment découvertes en Belgique. *Bull. Acad. roy. belge.* **19**, 110–132.

Westendorp, G. D. (1861). Sur quelques Cryptogames nouvelles, ou inédites pour la flore belge, septième notice. *Bull. Acad. roy. belge.* (2*me sér.*), **11**, 644–660.

Yarwood, C. E. (1941). Diurnal cycle of ascus maturation of *Taphrina deformans*. *Amer. J. Bot.* **28**, 355–357.

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CAMPHOR-INDUCED *GIGAS* FORMS IN *NEUROSPORA*

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(With 3 Text-figures)

Gigas strains with microconidia larger than the standard have been obtained in *Neurospora crassa* after camphor treatment. Such types are unstable, showing a tendency to revert to the standard condition.

An ascus with approximately the tetraploid number of chromosomes was observed in a cross between two unstable *gigas* strains and a diploid and triploid ascus in a cross between a *gigas* strain and the control.

In crosses involving *gigas* types, asci with more than 8 ascospores were found (but not regularly 16-spored asci as in *Aspergillus* diploids). There were also many empty perithecia. These were not found in the control crosses. Irregular pairing and segregation in triploid asci might lead to ascus abortion and empty perithecia. These observations therefore accord with the view that the *gigas* forms are diploid.

A cross between 2 unstable *gigas* strains gave 7 single ascospore cultures of mating type *A*, of which 2 were highly fertile and presumably haploid and 5 sterile and presumably diploid; and 4 of mating type *a*, of which 2 were probably diploid and 2 haploid. In addition, there were 7 neutral cultures which did not react with either mating type.

Introduction

It has been possible to obtain *gigas* forms of *Penicillium notatum* after treatment with camphor. Such strains have conidia, which are uninucleate, larger than those of the standard strain, the relative diameters being 1 : 1·2. These *gigas* strains have larger nuclei than the standard and the presumption is that they are diploid. A study of variation in one of these strains supports this view (Sansome, 1949). However, it has not been possible to make chromosome counts or to study inheritance after crossing since there is no known sexual stage in *P. notatum*. Consequently, it seemed advisable to try to obtain *gigas* strains in some species with a controllable sexual reproduction. To identify diploid strains a fungus must have spores with a fixed number of nuclei, generally one. Wild-type *Neurospora* has multinucleate conidia showing a great variation in size and number of nuclei, and is therefore unsuitable. A form of *Neurospora* (obtained from Dr Garnjobst) producing no macroconidia, but an abundance of uninucleate microconidia was used in the experiments. Later, a *fluffy* type producing microconidia on wetting was used also.

Methods

Large slanted tubes, each containing 20 ml. of malt agar were used. For treated cultures, 50, 75 or 100 mg. of camphor were added to the melted agar. Later, 60 mg. was chosen as the most suitable amount. In the first

experiment inoculations were made with a piece of mycelium, whilst later a suspension of microconidia was spread over the whole surface of the slant. Growth was inhibited for a long time in the treated tubes. Later a stunted growth appeared and as soon as microconidia could be obtained they were plated, and single germinating conidia were transferred into tubes. Control cultures were similarly plated out. Sometimes, because of the great delay in growth of the treated tubes, subcultures from the original controls were used as controls for the treated cultures.

Because of the inhibiting effect on growth of the camphor treatment it was decided to shorten the period of treatment. This was done by wrapping camphor in cellophane, placing it above the plug of the tube and covering the plug with aluminium foil. The cellophane packet of camphor could be removed when desired. When this improved method was used the original cultures were treated for 4 days before conidia were taken, and after inoculation with conidia a further 4-day treatment was given. In one experiment 20 mg. of camphor was added to the medium to re-inforce the effect of the camphor plugs.

As soon as possible the single spore cultures obtained after camphor treatment, together with the controls, were examined for microconidial size, camera lucida drawings being made with a × 10 eyepiece and a × 100 or × 120 objective.

Results of camphor treatment

Microconidial development was not good under the conditions of the experiment so that it was sometimes difficult to obtain sufficient microconidia for plating and for determinations of conidial size. However, some cultures were obtained with conidia larger than the standard as shown in Table 1. Of the control cultures, 57 single-conidial isolations all had conidia of standard size, whereas of the treated cultures 89 had conidia of average size, 13 had larger conidia and 23 had conidia of mixed sizes.

Table 1. *Results of camphor treatment of* Neurospora crassa

Culture	Treatment	Number of single microconidial cultures with conidia			
		Standard	Larger	Mixed	Total
Exp. 1. *mic fluffy*	75 mg. camphor, 20 ml. agar	20	—	—	20
	Control	20	—	—	20
Exp. 2. *mic fluffy*	60 mg. camphor, 20 ml. agar	15	8	7	30
	Control	27	—	—	27
Exp. 3. 1^{6} *non-mic fluffy*	20 mg. camphor, camphor plug	41	5	6	52
	Control	2	(2)*	—	2
1^{2} *mic fluffy*	20 mg. camphor, camphor plug	13	—	10	23
	Control	8	—	—	8
Totals	Treated	89	13	23	125
	Controls	57	—	—	57

* Cultures producing macroconidia.

It seems evident that *gigas* forms can be obtained in *Neurospora* after camphor treatment as in *Saccharomyces* (Bauch, 1941), *Penicillium* (Sansome, 1946) and *Aspergillus* (Roper, 1952).

Instability of types with larger conidia

In contrast with *Penicillium*, where cultures could usually be classified as standard or *gigas*, there are in *Neurospora* a number of cultures with conidia of mixed type. If the larger conidia are diploid, as is believed, the presence of mixed conidia in a single conidial culture must be due to mutation during the development of the culture. Thus either the culture started as haploid and for some reason, possibly a delayed effect of the camphor treatment, one of its nuclei became diploid during development, or the culture started as diploid and one or more of its nuclei became haploid during development. Some single conidial isolations from one of the types with larger conidia were made but unfortunately microconidial size was not determined for these cultures. However, the evidence is in favour of the second hypothesis. When the cultures with larger conidia were subcultured and the conidial size of the cultures was determined, it was found that some of the cultures had reverted to the development of conidia of the standard size. For example, one culture recorded as having larger conidia gave 10 subcultures with mixed conidial sizes, 2 standard and 1 mainly standard with a few larger conidia. Subcultures of types recorded as having larger conidia had mixed or standard size conidia. It seems, therefore, that the larger conidial types are unstable. If they are diploid it appears that reduction of chromosome number occurs during growth, and haploid nuclei tend to replace diploid during colony growth. This instability of the *gigas* form in *Neurospora* contrasts with the behaviour in *Penicillium notatum* strain 1978B, in which *gigas* strains have been maintained in culture for over 7 years. However, one or two reverted subcultures have been obtained from this strain. Moreover, in the case of *P. chrysogenum* Q176, *gigas* forms were much less stable, reverting frequently to the standard type (Sansome, unpublished).

The difference in stability of the *gigas* forms in *P. notatum* and *Neurospora crassa* may be due not to a difference in the original back-mutation rate, but to a difference in the propagation- and survival-rates of the two types of nuclei in the two species.

Production of *gigas* strains on mating types *A* and *a*

It was desired to produce *gigas* forms of both *A* and *a* mating types so that they could be crossed, therefore Miss Garnjobst's line which was *microconidial, fluffy, a* was crossed with a wild type *A*. Of 26 single ascospore cultures, isolated from the cross, 15 were *non-fluffy* and produced macroconidia, and the remainder were *fluffy-microconidial* or *fluffy*. Two of these cultures were selected for treatment, 1^2 which was *microconidial, fluffy a* and 1^6 which was *non-microconidial, fluffy A*. It was felt that the greater difficulty of getting microconidia from cultures derived from 1^6 would be compensated for by the increase in fertility when crossing and by the segrega-

tion of the microconidial factor. One set of single ascospore cultures from 1^2 by 1^6 gave 12 *fluffy* to 13 *fluffy microconidial* cultures.

The camphor-treated cultures of 1^6 gave single conidial isolations comprising family 2, untreated cultures; single conidial isolations comprising family 4 from a treated culture. From 1^2 were derived, family 3, consisting of a single conidial isolation from an untreated culture and family 5, single conidial isolations from a camphor-treated culture.

The control culture for 1^6, when plated, gave 22 cultures of the parental type and 8 mutant cultures with reduced growth and of a pinkish brown colour. Two of the mutants had a few macroconidia when examined for conidial size and the remainder had no detectable conidia. Two of the *fluffy* cultures had conidia of standard size, and the remainder had too few microconidia for their size to be recorded (Table 1, Exp. 3). It is worthy of note that the determination of conidial size was always much more difficult in this *Neurospora* material than in the *Penicillium* strains previously studied because of the relative scarcity of conidia.

Intercrossing of *gigas* and standard types

The following cultures were used in crosses:

4^3 *fluffy non-microconidial A*, control.
5^2 and 5^1 *fluffy microconidial a*, control.
2^{13} *fluffy A*, conidia of mixed size.
3^{10} *fluffy microconidial a*, conidia of mixed size.

It may be noted that 3^{10} had a higher proportion of large conidia than 2^{13}. In discussing these crosses it will be assumed that the cultures with larger conidia are diploid, and those with conidia of mixed size are diploid-haploid heterokaryons.

In the crosses involving haploid and diploids there was a large number of empty perithecia. Crosses involving 3^{10}, which had a higher proportion of larger conidia than 2^{13} according to direct observation, were especially noticeable for the presence of empty perithecia. It is thought that the empty perithecia may represent the haploid-diploid combinations.

An attempt was made to measure the fertility of the crosses by recording the number of regular 8-spored asci, and the asci with more or less than 8 spores in individual perithecia. Another estimate of fertility can be made by counting the total number of asci recorded for number of spores in each perithecium. This estimate must be regarded with caution since the number of recordable asci is dependent upon the stage of development of the perithecium as well as upon the total number present.

The results are summarized in Table 2. In the cross $3^{10} \times 2^{13}$, one perithecium was much more fertile than the rest, so the results are given including and excluding this perithecium. The cultures 3^{10} and 2^{13} may be considered to be haploid-diploid heterokaryons, 2^{13} having a higher proportion of haploid nuclei, according to observations on microconidial size. It is likely, therefore, that 2^{13} functions predominantly as a haploid in crosses, whereas 3^{10} may function predominantly as a diploid. In

previous experiments on heterokaryons both types comprising the heterokaryon could be found among single ascospore cultures from one perithecium (Sansome, 1947). More than one pair of nuclei may therefore initiate the ascogeneous nuclei, which means that a perithecium may contain diploid and triploid asci when a haploid-diploid heterokaryon is crossed with a haploid control. When two such heterokaryons are intercrossed there is a possibility of obtaining diploid, triploid and tetraploid asci. If one attributes the sterile perithecia to the haploid-diploid combinations that does not necessarily exclude the possibility of obtaining triploid asci in the presence of diploid asci.

Table 2. *Fertility of crosses*

Cross	No. of perithecium recorded	No. of asci per perithecium	No. asci recorded	Av. asci per perithecium	8-spored asci	Less than 8-spored asci	Greater than 8-spored asci	Percentage of regular asci
$4^3 \times 5^2$	21	9–52	469	22·3	263	206	—	56
$2^{13} \times 5^1$	14	6–25	178	12·7	128	50	—	72
$3^{10} \times 4^3$	8	3–22	75	9·4	19	54	2	25
	7*	3–11	53	7·6	8	44	2	15
$3^{10} \times 2^{13}$	8	2–14	58	7·3	18	34	6	31

* Results excluding one aberrant perithecium.

The control cross 4^3 by 5^2 gave 263 regular asci to 206 asci with less than 8 spores in 21 perithecia, that is 56 % of regular asci. There were no asci with more than 8 spores. The cross 2^{13} (haploid-diploid) $\times 5^1$ gave 128 regular asci and 50 with less than 8 ascospores, that is, 72 % of regular asci. There were no asci with more than 8 ascospores. The high fertility as measured by the proportion of regular asci indicates that 2^{13} was functioning mainly as a haploid, most of the developing asci being diploid. The average number of asci recorded per perithecium was 22·3 in the control and 12·7 in the $2^{13} \times 5^1$ cross. If this indicates a difference in total number of ripe asci produced and not simply a difference in the number ripe at any one time, then the reduction in number may be due to the formation of triploid asci which fail to develop. Such a reduction in total number of asci might make the remaining asci more fertile by reducing physiological causes of sterility.

The cross 3^{10} (haploid-diploid) $\times 4^3$ (haploid) gave 19 regular to 56 irregular asci or 25 % of regular asci in 8 perithecia. If one perithecium more fertile than the rest is excluded the results are 8 regular to 46 irregular asci or only 15 % of regular asci. This cross was therefore considerably less fertile than the control. Moreover, two of the irregular asci had more than 8 ascospores, a condition not observed in the controls. It seems possible, therefore, that some of the asci are triploid. These results are confirmed by cytological observation, in which one ascus was found to have more than 7 pairs of chromosomes, probably 21 chromosomes.

The cross $3^{10} \times 2^{13}$ (haploid-diploid $\times$ haploid-diploid) gave 18 regular to 40 irregular asci from 8 perithecia or 31 % of regular asci. There were a great number of empty perithecia. Moreover, 6 of the irregular asci had

more than 8 ascospores (Fig. 1). It seems probable therefore that tetraploid and possibly triploid asci are produced in this cross. The occurrence of tetraploid asci was confirmed by cytological observations. Thus, in crosses involving diploid-haploid heterokaryons, there was always a number of empty perithecia, and the average number of recordable asci per perithecium was less than in the controls. The percentage of regular asci was less in crosses involving 3^{10}. The results from the 2^{13} combinations were somewhat anomalous. In the cross between 2^{13} and the haploid control, the number of recorded asci was reduced as compared with the control haploid-haploid cross but the percentage of regular asci was high,

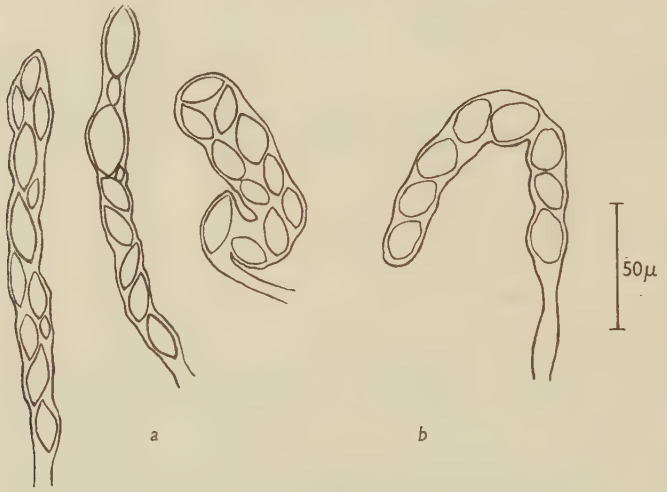

Fig. 1. *Neurospora crassa*. Irregular asci. (See text.)

72 % as compared with 56 % in the control. The difference between the behaviour of 3^{10} and 2^{13} may be accounted for in various ways. In the first place, 2^{13} reverts readily to the haploid condition, and it may have done so in the cross examined. There is no conclusive evidence that polyploid asci were obtained from this cross. Empty perithecia were found however, and the number of recorded asci was less than in the control cross. The difference between the crosses may have been due simply to a difference in the proportions of diploid and triploid asci formed. If a high proportion of the triploid asci abort, the fertility of the remaining diploid asci may increase as a result of reduced competition for the food supply. On the other hand, the surviving triploid asci might be expected to add to the numbers of irregular asci. Therefore the final effect on the percentage of regular to irregular asci would be determined by a balance between these two opposite tendencies, and would be correlated with the proportions of haploid and diploid nuclei taking part in the cross. It is also possible that the viability of *Aaa* asci might be different from that of *AAa* asci.

The observation of asci with more than 8 ascospores in crosses involving 3^{10} is in favour of this culture containing diploid nuclei.

Cultures from the cross $3^{10} \times 2^{13}$ (haploid-diploid × haploid-diploid)

Nineteen single ascospore cultures were obtained from the cross $2^{13} \times 3^{10}$. Such cultures might be expected to have come mainly from diploid and tetraploid asci and to be haploid and diploid with the possibility of occasional aneuploids from the tetraploid asci.

Of the cultures, 7 appeared to be normal *fluffy*, 2 were *fluffy* but rather thin and the remainder had a scanty growth-habit and were possibly *microconidial fluffy*.

The cultures were crossed with tester strains 4^3a and 5^2A in order to determine their mating type. Amongst the diploids, cultures heterozygous for the mating type factors might be expected to occur. The behaviour of such types could not be predicted. They might react with one or other of the mating types, with both or with neither. It was thought that such cultures might be self-fertile because of possessing both mating type factors, or as a result of somatic reduction producing haploid nuclei of both types, or as a result of somatic crossing-over producing *AA* and *aa* nuclei. However, none of these 19 cultures has been self-fertile while under observation.

Table 3. *Fertility of cultures from diploid-haploid × diploid-haploid*

No. of culture	No. of 8-spored asci	No. of irregular asci	Total asci	Percentage regular	Conidial size	Possible type
		I. Cultures which reacted with 4^3a				
6^1	15	7	22	68	—	Haploid
6^2	2	10	12	17	Mixed	Diploid
6^3	2	12	14	14	Mixed	Diploid
6^4	Not recorded; very sterile			—	—	—
6^6	16	8	24	67	Standard	Haploid
6^8	Not recorded; very sterile			—	—	Diploid
6^{10}	Not recorded; very sterile			—	Mixed	Diploid
		II. Cultures which reacted with 5^2A				
6^5	15	5	20	75	Standard	Haploid
6^{13}	75	151	226	33	Mixed	Diploid
6^{16}	28	11	39	72	Standard	Haploid
6^{18}	14	22	36	39	Mixed	Diploid

Seven cultures reacted with 4^3 and were therefore of mating type *A*. Of these, two, 6^1 and 6^6, gave 68 and 67 % of regular asci as shown in Table 3 and were therefore believed to be haploid. This was confirmed in the case of 6^6 by observations on microconidial size. Two cultures, 6^2 and 6^3, were less fertile on crossing, with 17 and 14 % of regular asci and had some larger conidia as shown in Fig. 2. They are therefore presumed to have come from diploid spores. Crosses involving the three remaining cultures, 6^4, 6^8 and 6^{10}, could not be recorded for the percentage of regular asci but they were very sterile. Microconidia were not obtained from 6^4 and 6^8, but 6^{10} had microconidia of mixed sizes (see Fig. 2). Four cultures reacted with 5^2 and were therefore of mating type *a*. Of these, two, 6^5 and 6^{16} gave relatively fertile combinations with 75 and 72 % respectively of

regular asci and were therefore probably from haploid spores. This was confirmed by observations on microconidial size of 6^{16}. The other two cultures, 6^{13} and 6^{18} had microconidia of mixed sizes and gave 33 and 39 % of regular asci. They are assumed to be derived from diploid spores. In the crosses involving 6^{13} and 6^{18}, asci containing more than 8 ascospores were observed. If these are triploid asci, then *Aaa* asci must have formed spores. In the original crosses involving diploid nuclei, such asci were found only in the *AAa* combination. The failure to find them in the $2^{13} \times 5^{1}$ cross may have simply been due to the high proportion of haploid nuclei in 2^{13}.

Some empty perithecia were noticed in crosses involving 6^{2}, 6^{8}, 6^{10} and 6^{13}.

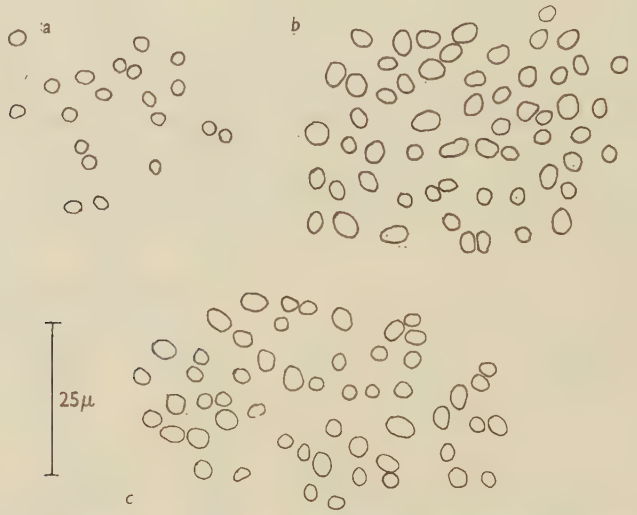

Fig. 2. *Neurospora crassa.* Diploid and haploid microconidia. (See text.)

In the combination $6^{4} \times 4^{3}$ small perithecia were produced but no spores were found. In the remaining 7 cultures 6^{7}, 6^{11}, 6^{12}, 6^{14}, 6^{15} and 6^{19} no mating type reaction was obtained after repeated attempts with both tester types.

The nature of the sterile cultures

The cultures which did not react with either of the tester strains may be diploid and neutral because they contain both mating type factors. It had been supposed that such types would be self-fertile due to segregation of the 2 types either by the reduction to the haploid condition or by somatic crossing-over. However, no self-fertile cultures have been found. There remain several possible explanations. These cultures may be sterile because the segregation of *A*, *a* is suppressed in some way, possibly by association with lethal factors, or they may be aneuploids. Segregation by somatic crossing-over might be expected to be infrequent for these genes since they are situated near the centromere, 10·8 units according to Lindegren (*J. Genet.* **32**, 243–256). Unfortunately, no microconidia have been obtained

from these cultures and though one culture gave a few macroconidia it was not a typical macroconidial type. If these neutral cultures are *Aa* types it may be that the absence of microconidia is associated with the neutrality of the cultures and indicates a spermatial origin of the microconidia. The microconidia have been called spermatia and have been regarded as having had an original function in bringing about diploidization of the ascogonium. However, they are capable of development into new mycelia so they have generally been regarded as vegetative spores.

It was thought possible that the usual reaction between haploid-diploid heterokaryons and *A* and *a* strains might be due to a reaction between the haploid nuclei of the heterokaryon and the tester strains. If this is so and if diploids are formed which do not give haploid nuclei, such diploids might be sterile when tested with haploid tester strains but they might react with diploid tester strains. Accordingly, the neutral cultures 6^{7} and 6^{12} were crossed with 6^{13}, believed to be *aa* and with 6^{2}, believed to be *AA*. The results were negative except that 6^{12} gave empty perithecia with 6^{2}. Another neutral, 6^{19} was crossed with 6^{4} *AA*?, 6^{8} *aa*? and 6^{17} neutral, with negative results. These results cannot be held to support the view that the neutral types are stable *AA* or *aa* diploids. They might be aneuploids but if so they might have been expected to give abortive perithecia rather than no reaction at all. In one combination only was this result obtained.

It seems most probable that the neutral cultures, as first suspected, represent the *Aa* diploids. If this is so, the absence of evidence of reversion to the haploid condition, such as occurred in the suspected *AA* and *aa* diploids, may be a result limited to this particular experiment or it may be a special property of the *Aa* combination. Future work on diploid strains of *Neurospora* should be of especial interest from the aspect of the behaviour of the mating type factors.

Summarizing the results of the experiments with family 6, there were 2 haploid *A*, 2 haploid *a*, 5 possible diploid *AA* and 2 possible diploid *aa*, together with 7 neutrals. If the neutral cultures are *Aa*, then there were 7 *Aa*:7 (*AA* plus *aa*) cultures which is the ratio expected on the basis of random segregation. Therefore the number of neutral types is in accordance with the hypothesis that they represent the *Aa* combinations, although the numbers involved are too low to be significant.

Cytological observations

Perithecia were cooled in the refrigerator for 2 hr. and fixed in acetic alcohol (3 alcohol : 1 acetic acid). The perithecia were mordanted in iron as recommended by Fincham (1949), then put into a watch-glass with weak aceto-carmine for 0·5–4 hr. Temporary aceto-carmine squashes were made by pressing individual perithecia so as to squeeze out the contents and removing the perithecial wall before putting on the cover-slip. Preparations of asci at various stages and of ascogenous hyphae were obtained in this way. The preparations were improved by gentle heating and by further pressure. In some cases perithecia were fixed in Nawaschin and stained by the Feulgen procedure with 20 min. hydrolysis. After staining

the asci were squeezed out of the perithecia and mounted in 45 % acetic acid. Attempts to use aceto-orcein instead of aceto-carmine were unsuccessful. This was in accordance with the experiments of McClintock (1945). However, Singleton (1953) reports that 2 % orcein (Eastman Synthetic, Cert. No. F Or-2) in 60 % acetic acid is satisfactory.

The cytological results are scanty in relation to the time spent on them for two main reasons. First, in crosses involving suspected diploid nuclei there are many empty perithecia and perithecia containing a reduced number of asci. Secondly, it was difficult to get a good differential staining of the nucleus and cytoplasm.

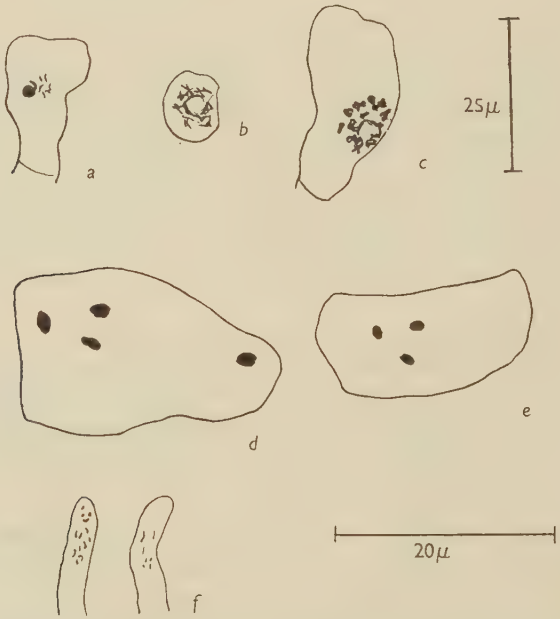

Fig. 3. *Neurospora crassa*. Cytology of ascus. (See text.)

Fig. 3*a–c* shows three ascus nuclei in which the chromosomes are identifiable. Fig. 3*a* is from a 5^{1} (haploid) $\times$ 2^{13} (haploid-diploid) cross and shows 7 paired chromosomes. McClintock (1945) observed that the chromosomes in *Neurospora* begin to pair in a contracted condition. This observation was confirmed by Singleton (1953). It seems that in *Neurospora* the chromosome pairing begins when the chromosomes are contracted and continues as the chromosomes elongate. Pairing is usually complete by the time the chromosomes have reached a length two or three times their original size. Elongation proceeds and the nucleus and nucleolus enlarge until a stage similar to ordinary pachytene is reached. Afterwards the chromosomes contract during the diplotene to metaphase stages. Fig. 3*a* apparently corresponds to the early pre-pachytene stage of pairing. Fig. 3*b* shows a nucleus from the 3^{10} (haploid-diploid) $\times$ 4^{3} (haploid) cross at a presumably later stage when the chromosomes are more extended.

This is approximately triploid. Fig. 3*c*, from the 3^{10} (haploid-diploid) $\times$ 2^{13} (haploid-diploid) cross, is from a temporary Feulgen squash. The nucleus appears to be tetraploid. The ascus in Fig. 3*b* is shorter than expected from the stage of development of the chromosomes and nucleolus, but the relation between nuclear development and ascus development may be different in polyploid asci. The evidence from haploid-diploid crosses indicates that triploid combinations are highly infertile.

Two nuclei were observed at metaphase in the ascogeneous hyphae of the combination 2^{13} (haploid-diploid) $\times$ 5^{2} (haploid). One nucleus had 7 chromosomes, the other 14 (Fig. 3*f*).

Measurements were made of the nuclei in the ascogeneous hyphae in the control 4^{3} by 5^{2} and in the 6^{18} (diploid *aa*?) $\times$ 6^{8} (diploid *AA*?) cross (Figs. 3*e-d*). The nuclei in the latter combination were longer than in the control, the ratios of the lengths being 1·3 : 1. This is the order of difference to be expected if the nuclei of 6^{18} and 6^{8} are diploid, the controls being haploid.

Discussion

Gigas forms of *Neurospora crassa*, if they are diploid as the evidence suggests, could have arisen by failure of mitosis, leading to a doubling of the chromosome number.

Roper (1952) has isolated diploids in *Aspergillus* which, since they were heterozygous, must have resulted from nuclear fusion. The frequency with which such diploids was obtained ranged from 3·4 to 271 per 10^{6} (Pontecorvo & Roper, 1953). Since the technique of Roper selects only the heterozygous diploids, and since homozygous diploids would be expected to be formed also as the result of fusion of similar nuclei, the actual frequencies of diploids resulting from nuclear fusion must be higher than the observed frequencies of heterozygous diploids. However, the frequency of diploids in *Neurospora* is very high (2 out of 30 in one experiment) and indicates that some factor other than nuclear fusion is also operating. Since failure of nuclear division resulting in chromosome doubling has been observed to occur in higher plants following treatment with camphor and other chemicals, it seems reasonable to assume that such a failure of division accounts for the chromosome doubling in this case also.

By using two strains with complementary deficiencies on homologous chromosomes it might be possible to synthesize a more stable diploid by the Roper method. However, in view of the difficulty of establishing vigorous heterokaryons involving the mating type factors (Gross, 1950), it might not be possible to obtain diploids heterozygous for the mating type factors directly by the Roper method. It is the behaviour of these factors in particular which merits further investigation in *Neurospora*. Colonial forms such as *Aspergillus* and *Penicillium* are more favourable for the study of recombination due to mitotic crossing-over and reduction to the haploid condition, but since they are asexual or homothallic, mating type factors cannot be studied in these species. During the present experiments, self-fertile cultures which might have been expected to occur as the result of somatic reduction in *Aa* diploids were not found. This accords with the observations of Pittenger (1954) on pseudo-wild types, believed to be

disomics in *Neurospora crassa*. These disomics were usually heterozygous for the segregating genes on the extra chromosome, but cultures disomic for the chromosome carrying the mating type factors always gave one or other of the mating reactions, never both, except that a few cultures were completely sterile. If the sterile cultures in the present experiments represent the cultures heterozygous for the mating factors, then the fact that they have not so far been observed to become self-fertile requires explanation. Further work may result in the production of diploid cultures which will react with both mating types and be self-fertile.

REFERENCES

Bauch, R. (1941). Experimentelle Mutationsauslosung bei Hefe und anderen Pilzen durch Behandlung mit Camphor, Acenaphthene und Colchicin. *Naturwissenschaften*, **29**, 503–505.

Fincham, J. R. S. (1949). Chromosome numbers in species of *Neurospora*. *Ann. Bot., Lond.*, NS, **13**, 23–28.

Gross, Samson R. (1950). Heterokaryosis between opposite mating types of *Neurospora crassa*. *Biol. Bull., Woods Hole*, **99**, 331–332.

Mcclintock, B. (1945). *Neurospora*. I. Preliminary observations of the chromosomes of *Neurospora crassa*. *Amer. J. Bot.* **32**, 671–678.

Pittenger, Thad. H. (1954). The general incidence of pseudo-wild types in *Neurospora crassa*. *Genetica*, **39**, 326–342.

Pontecorvo, G., Tarr Gloor, Elizabeth & Forbes, E. (1954). Analysis of mitotic recombination in *Aspergillus nidulans*. *J. Genet.* **52**, 226–237.

Pontecorvo, G. & Roper, J. A. (1953). Diploids and mitotic recombinations. *Advanc. Genet.* **5**, 218–233.

Pontecorvo, G. & Sermonti, G. (1953). Recombination without sexual reproduction in *Penicillium chrysogenum*. *Nature, Lond.*, **172**, 126.

Roper, J. A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia*, **8**, 14–15.

Sansome, Eva R. (1946). Induction of *gigas* forms in *Penicillium notatum* by treatment with camphor vapour. *Nature, Lond.*, **157**, 843.

Sansome, Eva R. (1947). The use of heterokaryons to determine the origin of the ascogeneous nuclei in *Neurospora crassa*. *Genetica*, **24**, 59–64.

Sansome, Eva R. (1949). Spontaneous mutation in standard and *gigas* forms of *Penicillium notatum* strain 1249 B21. *Trans. Brit. mycol. Soc.* **32**, 305–314.

Singleton, J. B. (1953). Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. *Amer. J. Bot.* **40**, 124 144.

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HAUSTORIA OF THE PERONOSPORALES*

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(With 5 Text-figures)

The somatic structure of some species of *Peronospora*, *Pseudoperonospora*, *Plasmopara*, *Bremia* and *Albugo* is described. The effect of the fungi on their hosts and the nature and importance of their haustoria are considered. Haustoria are not essential to all these parasites since they are not developed if sufficient food is supplied to the invading hyphae by diffusion from the host cells. The haustoria of *Peronospora* and *Pseudoperonospora* are hyphal branches which, when not impeded in their growth by the production of haustorial sheaths, are filamentous. Alterations in form and elaboration of haustoria, whether it is the development of branches or the formation of vesicles, is a result of host reaction. The haustoria of *Albugo*, *Plasmopara* and *Bremia* are more specialized, and these groups may therefore be considered to be more advanced as regards this feature than *Peronospora* and *Pseudoperonospora*. Some correlation has been found in certain species between the extent of development of haustoria and the supply of food, haustoria are simple in regions of the host where it is plentiful and they are more elaborate, presenting a more extensive surface where it is in short supply.

The length of life of the fungus mycelium in the host, whether it is relatively harmless and capable of perennation or whether it causes serious disease and rapid death, does not influence the form of haustoria. The vegetative structure and form of haustoria of these fungi is not correlated with their taxonomic position nor with the taxonomic position of their hosts.

INTRODUCTION

Information on the somatic structure of members of the Peronosporales is limited. Little is recorded as to the extent of their invasion of host cells and the frequency with which haustoria are produced. The occurrence and form of the haustoria is largely ignored in many descriptions. The literature shows that the same diagrams and drawings copied from de Bary (1863, 1887) and from Mangin (1895) were repeatedly reproduced and that these were not always accurate.

Classification of this group based on dimensions of conidia and conidiophores (both of which have been shown to be affected by external environmental conditions (Thung, 1926; Arens, 1928)) is not entirely satisfactory and it was thought possible that the form of haustoria might be of some taxonomic value. During the last four years about seventy members of the Peronosporales have been investigated from an anatomical viewpoint. Common species have been studied many times and at different times of the year. In all, some fifty species of *Peronospora*, two of *Pseudoperonospora*,

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eight of *Plasmopara*, three of *Bremia* and seven of *Albugo* have been examined (see Table 1). The descriptions include, also, all the information about haustoria of these forms which could be found in the literature.

Table 1. *Species examined*

Peronospora

P. ficariae (Nees) Tul. on *Anemone coronaria*, *Ranunculus ficaria*, *R. repens* (*P. ranunculi* Gäum.) and *R. bulbosus* (*P. ranunculi* Gäum.).
P. arborescens (Berk.) de Bary on *Papaver rhoeas* and *Meconopsis cambrica*.
P. affinis Rossm. on *Fumaria* sp.
P. parasitica (Pers. ex Fr.) Tul. on *Brassica oleracea* (cabbage and cauliflower), *Sinapis arvensis* (*P. brassicae*, Gäum.), *Capsella bursa-postoris*, *Diplotaxis muralis* (*P. diplotaxidus* Gäum.), *Rhynchosinapsis monensis*, *Cheiranthus cheiri* (*P. cheiranthi* Gäum.), *Matthiola* (Brompton stock).
P. niessleana Berlese on *Alliaria petiolata*.
P. dentariae Rabenh. on *Cardamine hirsuta*.
P. lepidii (McAlp.) Wils. on *Coronopus squamatus*.
P. alsinearum Casp. on *Stellaria media* (*P. media* Gäum.) and *Cerastium vulgatum* (*P. tomentosa* Fuckel).
P. arenariae (Berk.) Tul. on *Arenaria serpyllifolia* (*P. minor* (Casp.) Gäum.).
P. effusa (Grev.) Rabenh. on *Atriplex patula* (*P. minor* (Casp.) Gäum.) and *Chenopodium album* (*P. variabilis* Gäum.).
P. schachtii Fuckel on *Beta vulgaris* and *B. maritima*.
P. trifoliorum de Bary on *Medicago* sp. (*P. aestivalis* Sydow) *Trifolium pratense* (*P. pratensis* Sydow), *T. repens* (*P. trifolii-repentis* Sydow).
P. viciae (Berk.) Casp. on *Lathyrus pratensis* (*P. fulva* Sydow), *Pisum sativum* (*Peronospora pisi* Sydow), *Vicia sepium* (*P. sepium* Gäum.), *V. hirsuta* and *V. sativa* (*P. sativa* (Berk.) Gäum.).
P. potentillae de Bary on *Potentilla sterilis* (*Peronospora potentillae sterilis* Gäum.).
P. sparsa Berk. on *Rosa* cv. Tawny Gold.
P. violae de Bary on *Viola arvensis*.
P. conglomerata Fuckel on *Geranium molle*.
P. euphorbiae Fuckel on *Euphorbia paralias*.
P. rumicis Corda on *Rumex acetosa*.
P. debaryi Salmon & Ware on *Urtica urens*.
P. alta Fuckel on *Plantago major*.
P. chlorae de Bary on *Blackstonia perfoliata*.
P. grisea Unger on *Veronica beccabunga* and *V. persica*, *V. agrestis* (*P. agrestis* Gäum.), *V. serpyllifolia* (*P. verna* Gäum.).
P. sordida Berk. on *Scrophularia nodosa*.
P. lamii Braun on *Lamium purpureum*.
P. calotheca de Bary on *Asperula odorata* and *Sherardia arvensis* (*P. calotheca* var. *sherardiae* (de Bary) Rabenh.) and *Galium aparine* (*P. aparines* (de Bary) Gäum.).
P. destructor (Berk.) Casp. on *Allium ascolonicum*.

Pseudoperonospora

P. humuli (Miyabe & Tak.) G. W. Wilson on *Humulus lupulus*.
P. urticae (Lib.) Salmon & Ware on *Urtica dioica*.

Bremia

B. lactucae Regel on *Senecio vulgaris*, *Sonchus oleraceus*, *Lactuca sativa* and *Tragopogon minor*.

Plasmopara

P. viticola (Berk. & Curt. ex de Bary) Berl. & de Toni on *Vitus* sp.
P. densa (Rabenh.) Shroet. on *Rhinanthus minor* and *Odontites verna*.
P. nivea (Unger) Shroet. on *Anthriscus sylvestris* and *Aegopodium podagraria*.
P. pygmaea (Unger) Shroet. on *Anemone nemorosa*.
P. pusilla (de Bary) Shroet. on *Geranium pratense*.
P. epilobii (Rabenh.) Shroet. on *Epilobium parviflorum*.

Albugo

A. candida (Pers.) Kuntze on *Capsella bursa-pastoris*, *Cardamine hirsuta*, *Sisymbrium officinale*, *Alyssum saxatile*, *Aubretia* sp., *Arabis* sp.
A. tragopogonis (DC.) S. F. Gray on *Tragopogon minor*.

METHODS

The ordinary hand section was found to be adequate for the study of most fresh material. The most useful stain was Resorcin blue (B.D.H. 1 % (w/w.) in distilled water). Aniline blue in lactic acid was also used extensively. A freezing microtome was used for the study of material difficult to handle. The host tissues were broken up by this method, and this was often an advantage since the fungal hyphae withstood the treatment and a maceration was obtained.

Hand or freezing microtome sections of either fresh or preserved material were also treated with 'Pectozyme' solution (Fraymouth & Hawker, 1952). When the enzyme solution had acted on the host cells for 4–6 hr. a slight pressure on the coverslip was sufficient to separate them and show the relative positions of host and fungal cells. The maceration method of Mangin (1895), with some slight modifications, also gave good results with fresh, preserved or dried material.

Permanent microtome sections were made of several species where the host plant was sufficiently rigid.

All drawings were made with the aid of a camera lucida.

RESULTS

(1) The mycelium

The mycelium consists of coenocytic hyphae. The walls, as may be shown by the usual qualitative microchemical tests, consist chiefly of some form of cellulose. The hyphal walls are, however, very much more resistant to drastic maceration techniques than the cellulose cell walls of the higher plants. De Bary (1887) used the term 'fungal cellulose', thus indicating a difference in the properties of the material. According to the conclusions of Mangin (1895, 1910) and Thomas (1928) and the historical review by Foster (1949), the hyphal walls in this group may be regarded as consisting of cellulose in which other substances such as fats, fatty acids, hemicelluloses and materials of a pectic, protein and cutin nature are infiltrated in varying proportions. It was found during the course of this study that whereas the hyphae of most of the species tested with resorcin blue did not give a callose reaction, others stained in such a manner that a high proportion of callose could be assumed to be present in the hyphal walls.

The hyphae grow between the cells of the host tissues, branching in all directions, and they lie closely against the host cell walls. They are variable in width and form and take their shape from that of the intercellular spaces in which they occur. Where the intercellular spaces are small the hyphae are narrow, but when the host tissues are less compact the hyphae may branch profusely to fill the spaces or they may swell and widen considerably, often in one plane, becoming as much as six or seven times the width of an average hypha. The term 'palmette' formation was given to this type of development by Mangin (1895). In this investigation the palmette type of development has been noted in many instances and

particularly in species which do not appear to produce haustoria readily. As infection proceeds the cells of the host may be pushed apart from one another unless the plant is very rigid. Usually the epidermis remains normal in appearance even if the mesophyll cells inside are completely separated from one another. In other plants where the leaf structure is firm and the intercellular spaces small, the narrow hyphae wind between the cells without appearing to disrupt them so that in section it is difficult to distinguish the host and parasite without suitable staining. In some host leaves the invading hyphae tend to be confined to a layer or plane in the leaf where there is presumably, less resistance to their growth. Under certain physiological conditions adverse for the host, e.g. the drought during the spring of 1953, the hyphae of some downy mildews grow to form a solid mass of mycelium, almost a plectenchyma, under the epidermis of the host plant. These hyphae fill the air spaces under the stomata and lift the epidermis, forming small grey blisters. When weather conditions become more favourable, these hyphae grow out of the stomata below them and form conidiophores within a few hours. Millasseau (1928) has described this type of growth as occurring in *Pseudoperonospora humuli* 'when the tissues of the host are disorganizing'. He called it a 'coralloid' formation of the mycelium. The form taken by the hyphae is often quite different in petioles and stems from that shown in leaves of the same host plant. There is in general, much less branching and the hyphae are narrower and of more even diameter. The fungus is able, because of the structure of the host tissues, to take a straight course up or down the plant for considerable distances, growing vertically between the elongated cells of cortex or pith as the case may be.

There are several instances where the hyphae have been reported as growing for short distances outside the host tissues. Magnus (1894) found hyphae of *Peronospora parasitica* growing from one leaf to another in a bud of *Cheiranthus cheiri*, and the same fungus was observed by Thung (1926) growing in the same way in a head of cabbage. During this study, hyphae of *Peronospora grisea* were observed to have grown out of a stoma in a bud of *Veronica beccabunga* and to have formed a loose knot of mycelium from which a hypha had grown and infected a neighbouring leaf.

The length of life of the hyphae varies with the species of host and parasite concerned. In some leaves all hyphae appear to be turgid, full of living contents and food reserves and capable of further growth and branching, while at the same time conidiophores in close association are producing conidia in large numbers. In the leaves of other hosts the life of the hypha is shorter. Hyphae soon become attenuated and the contents become sparse and autolysed when the conidiophores mature. Often, hyphae disappear when oogonia are formed. In some species of *Peronospora* the hyphae are sealed by a plug of callose and further growth is by a side branch some distance from the plugged section. This sealing of the hyphae was noted by Mangin (1895) and he attempted to use it as a diagnostic character. In the leaf of *Anemone coronaria* infected with *Peronospora ficariae* the hyphae are very short-lived, and run for only the distance between three stomata and then turn out and grow through the next one and develop a conidiophore. At the base of each of these the hypha becomes

plugged with callose sealing it off from the rest of the fungus (Fig. 4D), while the conidia are still in process of formation. The walls of both host and fungus become dried, discoloured and brittle, but they still give a cellulose reaction with iodine and sulphuric acid. Haustoria are rarely seen.

Hyphae of several species have been found to live for long periods in parts of the host plant which persist from year to year, and there are now many records of downy mildews which perennate (Table 2). Leach (1931) has reported mycelium of *P. schachtii* inside the testa of viable beet seed. Host plants which have so far been reported as harbouring perennial mycelium are biennials or perennials which over-winter below or close to the ground, or they are annuals or ephemerals which can survive mild winter conditions. These hyphae live in the cortex or pith of stem, and root without causing serious damage to the host.

Table 2. *Members of the Peronosporales recorded as perennial*

(Data up to 1915 from Melhus (1915))

Species	Host	Reference
Phytophthora infestans	*Solanum tuberosum*	de Bary, 1861; Jensen, 1887; Melhus, 1913
P. cactorum	*Panax quinquefolium*	Rosenbaum, 1914
Plasmopara halstedii	*Helianthus diversicatus*	Melhus, 1913
P. pygmaea	*Hepatica acutiloba*	Stewart, 1910
P. viticola	*Vitis vinifera*	Istvanffi, 1904
Peronospora alsinearum	*Stellaria media*	Magnus, 1888
P. arborescens	*Papaver*	Yossifovitch, 1929
P. chlorae	*Blackstonia perfoliata*	Fraymouth, 1956
P. de Baryi	*Urtica urens*	Salmon & Ware, 1929
P. dipsaci	*Dipsacus fullonum*	Kühn, 1875
P. effusa	*Atriplex hortensis*	Magnus, 1888
P. effusa	*Spinacia oleracea*	Magnus, 1888
P. ficariae	*Ranunculus ficaria*	de Bary, 1876
P. ficariae	*R. fascicularis*	Melhus, 1913
P. grisea	*Veronica beccabunga*	Fraymouth, 1956
P. grisea	*V. hederaefolia*	Magnus, 1888
P. parasitica	*Brassica rapa*	Gardner, 1920
P. parasitica	*Lepidium virginicum*	Melhus, 1913
P. pulveracea	*Helleborus niger*	Klebahn, 1925
P. rumicis	*Rumex acetosa*	de Bary, 1876
P. schachtii	*Beta vulgaris*	Kühn, 1872
P. schachtii	*B. maritima*	Fraymouth, 1956
P. schleideni	*Allium* spp.	Murphy & M'Kay, 1926
P. viciae	*Vicia sepium*	Melhus, 1913
Pseudoperonospora humuli	*Humulus lupulus*	Ware, 1926
Albugo candida	*Capsella bursa-pastoris*	Melhus, 1913
A. candida	*Lepidium virginicum*	Melhus, 1913

The downy mildews are also capable of surviving as mycelium in the dead remains of the host plant, for example, in the debris of the ovary walls. As the seeds germinate they become infected by the parasite. The mycelium as a source of infection was noted by Yossifovitch (1929) in old fruits of *Papaver* which contained hyphae of *Peronospora arborescens*. During this investigation, *P. parasitica* has been observed to develop hyphae inside the fruit-wall of Brompton stock. They penetrated the layer of sclerenchyma on the inside of the pericarp, but were not, however, seen to enter

the testa. This fruit commonly lasts throughout the winter and may be a source of infection to seedlings which develop nearby.

The Peronosporales reproduce actively and reach serious proportions only when warm damp weather conditions prevail, conditions which favour the growth of young shoots on the host plants and which occur in this country in both spring and autumn.

(2) The haustoria

A haustorium is a branch of a hypha, and is composed primarily of similar material. Haustoria are usually multinucleate, and the density of the protoplasm and the contents vary as much as the contents of the hyphae. In *Albugo*, where the haustoria are much smaller than those of the other members of the Peronosporales, they were said by Wager (1896) to be uninucleate, and this has been confirmed. Haustoria vary very much, even when they occur in the same host cell. This variability accounts for contradictory reports which have been made on haustoria.

Penetration is usually by way of a canal of narrow bore made at the point of contact between the hypha and the cell-wall, but there are many examples of host plants, especially those with thickened and rigid walls, where the haustoria enter through natural pits already present in the cell-walls. Haustoria entering in this way have penetration tubes of varying width according to the structure of the host-wall. The iodine and sulphuric acid test for cellulose shows well the existence of thick and thin areas in the cellulose-walls of quite young cells of a normal leaf. Large simple pits are present in the older thickened cell-walls of cortex and pith. Branches of the hyphae which enter the host cells as haustoria are found at right angles to the mature hyphae in the majority of examples; they have originated near the tip of the young growing hypha, which has afterwards continued its growth leaving the haustorium as a side branch. Haustoria are often found, where it is obvious that the fungal hyphae have been held in check for a while by the host tissues. Thigmotropism, is however, not a complete explanation of haustorium formation in the Peronosporales which provide many examples of close contact between host and parasite without haustoria production.

The haustoria do not appear to penetrate the protoplast and they do not destroy the semipermeable properties of the protoplasmic membrane. Continued growth causes an invagination of the protoplast so that the haustoria are in very close contact with it. The branched or contorted type of haustorium has therefore a very extensive absorbing surface.

Experiments were carried out which demonstrate that haustoria are not within the protoplast but are completely surrounded by it. The method is a modification of plasmolysis methods used by Thatcher (1939). Sections were stained with Neutral Red (200 p.p.m. in tap water), then irrigated with calcium chloride solutions of suitable strengths to bring about gradual plasmolysis of the cell contents. Cells containing haustoria in favourable positions were observed and drawn. As plasmolysis proceeded, the coloured protoplasts contracted away from the haustoria, showing them to

be entirely free from the protoplast. Subsequent irrigation with tap water usually resulted in a return to the original state and the process could be observed in reverse. Taken together with the fact that the sheaths of haustoria are a continuation of the host cell-wall, these results provide evidence that the protoplast is not directly injured by the development of haustoria within the living cell.

The sheath of the haustorium

As soon as the haustorium has penetrated the host cell-wall, reactions are set up between the host protoplast and the invading fungus. The wall of the haustorium becomes covered with a layer, moulded to its shape, produced by the host protoplast. This sheath is composed of the modified cellulose, callose, and unstained it may not be easily distinguishable from the fungal wall. It occurs in various physical states. Callose is a substance which may be mucilaginous, but it is always revealed by Resorcin blue. Eschrich (1954) has shown that bacterial action has the effect of causing callose to become mucilaginous, and it is perhaps due to the activities of the fungus that the physical nature of cell-wall material deposited by the protoplast on the surface of the haustorium varies. During periods of rapid growth of the haustorium, a layer in the form of a liquid or an easily diffusible gel surrounds the invader as soon as it penetrates the host wall. Changes in size due to osmotic effects may be observed under the microscope. This semi-liquid state is probably more common than might be supposed, as in section it is an ephemeral condition. When growth of the fungus is not vigorous the sheath may become a solid layer.

A thickened solid sheath often shows stratification, some layers being more opaque than others, the layers taking up stains to varying degrees. Some of these stratified sheaths were viewed under polarized light, but the layers were not distinguishable.

On a sudden increase in the growth rate of the fungus, this sheath is often burst, and it remains as a collar or layer of uneven thickness at the base (Fig. 2, C, E). Should the rupture occur in more than one place, the sheath may remain as pieces of tissue on the surface of the enlarged haustorium. There are some instances where a thick layer deposited all over the surface is subsequently broken by the further growth of the haustorium, and the pieces of the original sheath remain at the base or at other places on the surface (Fig. 2 A). This particular reaction is illustrated in the figure 'after Millardet' for *Plasmopara viticola* on *Vitis*. This picture of what is in reality an exceptional condition is copied in many text-books.

The variability in the extent and development of the sheath around the haustoria is explained if, as suggested by Rice (1927) for rusts, the sheath indicates the degree of resistance offered by the individual cells invaded.

It may be that the amount of nourishment available is the deciding factor determining the final form of haustoria. In a healthy leaf the haustoria are relatively simple and few in number; haustoria which develop in the pith or cortex of a stem, where there is likely to be less available food, are usually more elaborately developed (Fig. 1 A, A 2).

(3) Descriptions of haustoria

(*a*) *Haustoria of the genus* Peronospora *Corda*

The following descriptions of the haustoria, are grouped according to the families of flowering plants parasitized. The morphology of the haustoria supports the older concept of large group species of *Peronospora*, which may include a number of morphologically similar races. Except in a limited number of examples, this work does not support the considerable subdivisions of the old species suggested by Gäumann (1918*a*, 1923), but it does bring out the essential morphological similarities of strains on related host plants. It is logical, therefore, to follow the recommendation of the International Code of Botanical Nomenclature (Rec. 14A) that fungi differing only in host range within a group of closely related host plants should be given racial and not specific rank. Variations in the dimensions of conidia and conidiophores of one form on one host in different environments have in many instances been found to be greater than the differences which Gäumann considers to be specific.

DICOTYLEDONS

Ranunculaceae

Members of this family have been examined on very many occasions. Haustoria occur very seldom and then only in cells which are otherwise empty of contents and may be considered moribund. There is very close contact between the cells of the host and the hyphae, and in leaves the fungus grows between the cells and fills the intercellular spaces. The haustoria are very delicate, hyphal and branched and covered by a sheath of callose which is of uneven thickness though often thickest at the tips.

Peronospora ficariae (Nees) Tul.

On 1. *Anemone coronaria*. From Holcombe, Devon, 31 August and 8 September 1953, showed all stages of infection. The leaves were much dried and distorted. Hyphae permeated the tissues but were themselves short-lived, soon becoming sealed off by plugs of callose and becoming autolysed. Conidiophores were also soon sealed just inside the stomata from which they had emerged (Fig. 4D). Haustoria were very rare.

2. *Ranunculus repens* (*P. ranunculi Gäum.*). From Bristol district, April (several occasions); Slough, Berks, 27 April 1953; Cleeve, Som., 10 June 1953; Coniston Moor, Westmorland, 21 April 1954. Haustoria were filamentous, and branched and were seen in only three cells (Fig. 1C).

3. *Ranunculus bulbosus* (*P. ranunculi* Gäum.). From Bristol, April and May (many times); Berrow, Som., 14 May 1953; Windsor, Berks, 18 May 1954. A massive coralloid formation of hyphae, forming blisters under the lower epidermis was recorded in May 1953. Only three cells were seen to contain haustoria, two in the Windsor collection and one from Berrow. They were filamentous and branched, and were in moribund mesophyll cells. Oogonia were observed in specimens from Berrow.

4. *Ranunculus acris.* From Hambrook, Glos., 17 May 1954. Several cells contained haustoria. Oogonia were present.

5. *Ranunculus ficaria.* Material studied on many occasions from Bristol and Wotton-under-Edge, Glos., February to May, 1952 and 1953. Haustoria were rare but were seen in root and root-stock. Oogonia were usually present from February onwards.

Peronospora pulveraceae Fuckel on *Helleborus niger*

Klebahn (1925) states that it is perennial in the root stock and that the hyphae are intracellular. He figures hyphal haustoria in the root-stock but not in the leaf. Arx & Noordam (1951) state that the hyphae are primarily intercellular but may later penetrate the cell wall of the host. This suggests that when the host is almost destroyed the hyphae may grow through the cell-wall, forming filamentous haustoria as does *P. ficariae.*

Papaveraceae and Fumariaceae

The leaves contained small simple hyphal haustoria, best shown in macerations. In the petioles and stem the haustoria were larger and more extensive, often branched with a stalk.

Peronospora arborescens (Berk.) de Bary

On 1. *Papaver rhoeas.* From Bristol and Reading, Berks, June to August, 1952 and 1953. New infections and plants systemically infected were studied. Haustoria were usually not much developed in the leaves (Fig. 1 A), but were more common and fully developed in the collenchymatous cortex of the stem where the thick walls of the host possess large simple pits (Fig. 1 A 2). The haustoria were stalked and branched, the stalk being much thickened indicating an initial resistance by the host. They were multinucleate. Entry was either by a large cell wall pore or by a narrow channel made by the haustorium. Young filiform haustoria which had only just penetrated the mesophyll cells of the leaf were often found in pairs at a forking point of a hypha (Fig. 1, A 1). The form of the haustorium is therefore dependent upon the position and nature of the host cell invaded.

2. *Meconopsis cambrica.* Hawkshead Lancs., April, and Ambleside, Westmorland, June 1954. There were a few haustoria in the young leaves and petioles of both specimens.

Peronospora affinis Rossm.

On *Fumaria officinalis.* From Bristol and Reading, Berks, at different times during the season; Slough, Bucks, and Langport, Som., August 1953. Digitate haustoria, often knotted and oiled were in the leaf, petiole and stem (Fig. 1 B). Macerations of the leaves showed the presence of numbers of very small knob-like protrusions and some filiform outgrowths from the hyphae. The protrusions were of the order $3 \times 3\,\mu$, that is, not more than the width of the cell-wall of the host. They may be interpreted as either incipient or aborted haustoria, not large enough to have penetrated the host cells (Fig. 1 B 1). (Mangin (1895) figures these protrusions and labels

them as a second type of haustorium.) Oogonia were present in late summer.

Peronospora corydalis de Bary is described by Fischer (1892, p. 478). The haustoria are said to be thread-like, slightly branched and very scarce.

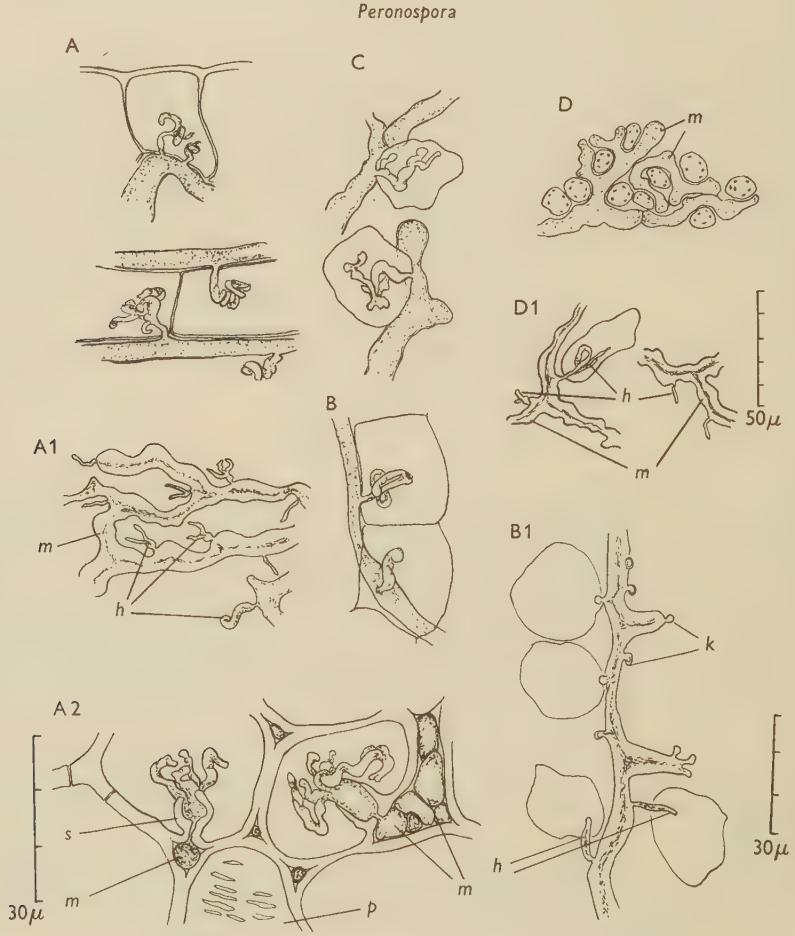

Fig. 1. A, *Peronospora arborescens* in leaf of *Papaver*. A1, the same macerated; (*h*) haustoria, (*m*) hypha. A2, T.S. *Papaver* stem; (*s*) remains of haustorial sheath; (*p*) pits in host cell-wall. B, *P. affinis* in leaf of *Fumaria*. B1, the same macerated; (*k*) incipient haustoria. C, *P. ficaria* in leaf of *Ranunculus repens*. D. *P. potentillae* hyphae in leaf of *Potentilla sterilis*; (*m*) hyphae. D1, the same macerated; (*h*) haustoria, (*m*) hyphae

Cruciferae

Haustoria are always present in the infected parts of members of this family. They are vesicular and are fairly constant in form and size for any particular host plant. For example, those found in *Matthiola* were always simple vesicles, those in *Capsella bursa-pastoris* much enlarged, lobed

vesicles. It was found that as an exception, the haustoria of *Peronospora lepidii* (McAlp.) Wils. were different from any other *Peronospora* in a Crucifer in being hyphal and branched.

Peronospora parasitica (Fr.) Tul. (*Peronospora brassicae* Gäum.)

On 1. *Brassica oleracea* (cabbage) from Bristol, May and June. Limited to small patches on the leaves. There were many haustoria which were large irregular vesicles. In one sample haustoria were all bilobed and regular in shape, suggesting that these features may depend upon the individual host concerned.

2. *Brassica oleracea* (cauliflower). From Wotton-under-Edge, Glos., July; infection very slight. Haustoria were numerous and were simple globose and uniform in size (Fig. 2A). The walls of the haustoria were surrounded in many instances by an unusual shining and granular layer similar to that figured by Millardet on *Plasmopara viticola.*

3. *Sinapis arvensis.* From Wraxall, Som., June; on young plants. The haustoria were large, bifurcating vesicles. Many were surrounded by sheaths which had been ruptured.

Peronospora parasitica (Fr.) Tul. (*P. matthiolae* Gäum.)

On *Matthiola* (Brompton stock). From Wotton-under-Edge, Glos., May to July (many occasions). Leaves, stems and fruits were infected and contained regular vesicular haustoria (Fig. 2B). The plants continued to flower, and a special study was made of the infected fruits. In a July sample the haustoria were so much thickened as to appear solid. Hyphae penetrated to the inside of the pericarp but no hyphae were found in the testas. Conidia were however shed at the same time as seeds were being liberated from the fruits.

Peronospora parasitica (Fr.) Tul. (*P. cheiranthi* Gäum.)

On *Cheiranthus cheiri.* From Bristol, February to November, 1953 and 1954. Vesicular haustoria were always present, their size varied with the size of the cell in which they developed. Young haustoria in the stem were always surrounded by a sheath (Fig. 2C).

Peronospora parasitica (Fr.) Tul. (*P. parasitica* Gäum.)

On *Capsella bursa-pastoris.* From the Bristol district, May to August. Haustoria were always present in the infected areas. They were vesicles, usually bilobed and of varying sizes, several in one cell (Fig. 2E).

Peronospora parasitica (Fr.) Tul.

On *Diplotaxis muralis.* From Berkhampstead, Herts, November 1953. Vesicular haustoria were present which were very regular in size and shape, irrespective of the size of the host cell (similar to those seen in *Matthiola*), some were enclosed in a thickened sheath, others were observed where the sheath was liquid inside (Fig. 2D). The volume of the sheath increased

when the sections were in water and collapsed on the addition of stain giving the peculiar egg-cup effect which is figured by Mangin (1895) around the haustorium of *Bremia*. Oogonia were present in the material.

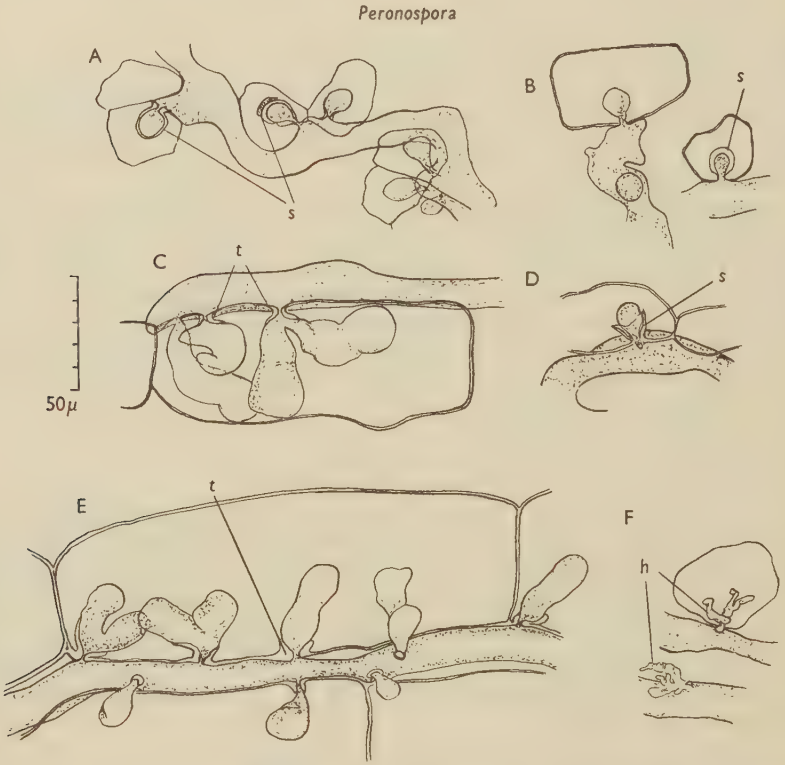

Fig. 2. A, *Peronospora parasitica* in cauliflower leaf; (*s*) sheath. B, *P. parasitica* in Brompton stock leaf; (*s*) sheath. C, *P. parasitica* in wallflower leaf; (*t*) thickening forming 'collar'. D, *P. parasitica* in *Diplotaxis muralis* leaf; (*s*) remains of sheath. E, *P. parasitica* in *Capsella bursa-pastoris* leaf, variation in haustorial shape; (*t*) thickening forming 'collar'. F, *P. lepidii* in *Coronopus squamatus*; (*h*) haustoria (hyphal and branched).

Peronospora parasitica (Fr.) Tul.

On *Rhynchosinapis monensis*. From Bristol, 13 December 1953; consisting of a slight infection of a few leaves. Hyphae and haustoria were scarce, the latter varied very much in size and shape, most of them were small vesicles coiled on themselves. Most of the haustoria were enclosed by sheaths, these varied, several consisting of a broken solid granular layer. The weather was very cold and thus unfavourable for growth.

Peronospora lepidii (McAlp.) Wils.

On *Coronopus squamatus*. From Wotton-under-Edge, Glos., June 1953 and Sempringham, Lincs (Herb. I.M.I. 53787). The haustoria were hyphal and branched, different from any other found on cruciferous hosts (Fig. 2 F).

Peronospora niessleana Berlese

On *Alliaria petiolata*. From Bristol and Slough, Bucks, April 1954. Haustoria were present in older leaves and in the petioles and stem. They were large vesicles which filled the cells in which they occurred. Oogonia were present in the leaves.

Peronospora dentariae Rabenh.

On *Cardamine hirsuta*. From Wotton-under-Edge, Glos., March. Vesicular bilobed haustoria were present in the leaves.

Violaceae

Peronospora violae de Bary

On *Viola arvensis*. From Wraxall, Som., and Frampton-on-Severn, Glos., June and July. After maceration many small branched and digitate haustoria were found. They usually consisted of a small bulbous base with slender finger-like projections (Fig. 3 F, F 1). Oogonia were seen in July.

Caryophyllaceae

The haustoria of *Peronospora* species, which are parasitic on members of this family, are usually of a simple tubular or hyphal type, with callose sheaths varying in thickness. Haustoria may not be necessary in young infections; many plants were found to be without them, although thoroughly parasitized.

Peronospora alsinearum Casp. (*P. arenariae* (Berk.) Tul.)

On *Arenaria serpyllifolia*. From Wraxall, Som., July 1953. Haustoria were not common except in areas of intense infection where the mesophyll cells were moribund. The haustoria were simple, thin-walled tubes and almost filled the cells. There was no evident host resistance inside the cells.

P. alsinearum Casp. (*P. tomentosa* Fuckel)

On *Cerastium vulgatum*. From Bristol, 1 May 1952 and Westmorland 2 June 1954. Haustoria present in leaf and stem were small and thread-like tubes, sometimes branched. There were often several in a cell.

Peronospora alsinearum Casp. (*P. media* Gäum.)

On *Stellaria media*. From Bristol district, May to October, 1953 and 1954. There were no haustoria in the leaves of specimens collected in the early part of the year, later in the season several were evident in every section, by October there were many substantial haustoria in many of the cells. The haustoria were stalked and digitate, thick-walled in the later collections. There was never any indication of host resistance at the point of entry. Oogonia were present in leaves and fruit wall in August.

Chenopodiaceae

The haustoria on members of this family were found to be hyphal, sometimes branched and coiling on themselves. The extent of development of the sheath varied considerably.

Peronospora

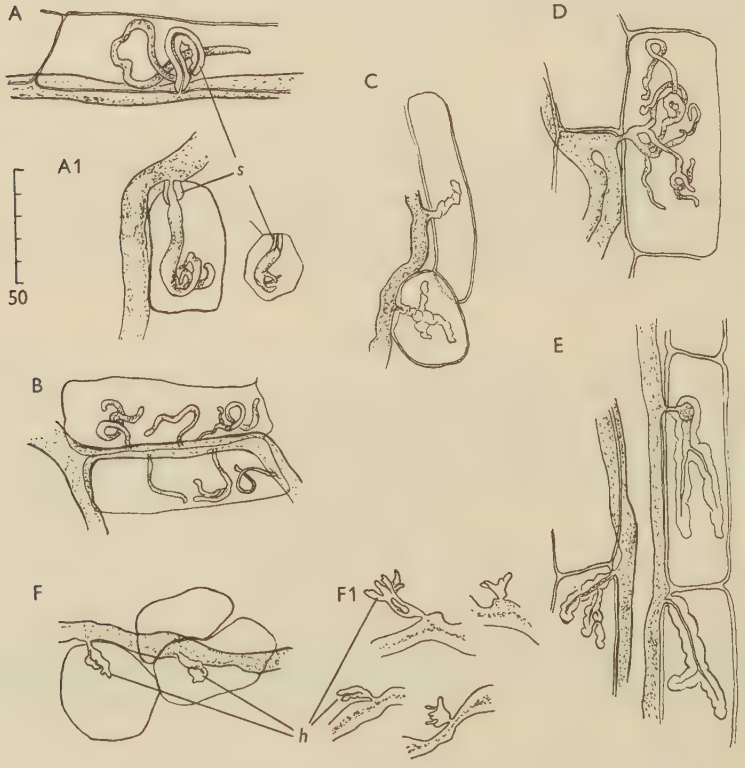

Fig. 3. A, A1, *Peronospora viciae* in *Vicia sepium*; (*s*) haustorial sheaths. B, *P. viciae* in *Pisum sativum* pericarp. C, *P. debaryi* in *Urtica urens* leaf. D, *P. calotheca* in *Asperula odorata* stem. E. *P. calotheca* var. sherardiae in *Sherardia arvensis* stem; (*s*) sheath. F, *P. violae* in *Viola arvensis* leaf; (*h*) haustoria. F1, the same macerated; (*h*) haustoria with bulbous bases and filiform extensions.

Peronospora effusa (Grev.) Rabenh. (*P. variabilis* Gäum.)

On *Chenopodium album*. From Cleeve, Som., 10 October 1949, 18 August 1952, 20 May 1953, and others. Filamentous and often branched haustoria were always present. When the hyphae were extensively developed the haustoria were numerous but thin-walled, thread-like in appearance. When there were not many hyphae in the leaf the haustoria were fewer in number and very much more substantial in appearance, suggesting a greater host resistance. Oogonia were present in October.

Peronospora effusa (Grev.) Rabenh. var. *minor* Caspary

On *Atriplex patula*. From Berrow, Som., 15 May and 28 June 1953; Lincoln, July 1953. All specimens contained a few cells with haustoria but they were not common. They were always stalked and branched coils and were thickened.

Peronospora effusa (Grev.) Raben.

On *Spinacea oleracea*. Haustoria, which were finger-like and branched, were reported by Richards (1939). Ikata and Yamauti (1941) illustrate a much branched hyphal haustorium of *Peronospora spinaciae*.

Peronospora schachtii Fuckel

On 1. *Beta vulgaris* (sugar beet). From Lincoln, 13 May and 11 July 1953; Wye, Kent, 5 June 1952. In the first collection, which consisted of mature leaves, the haustoria were few in number and were thick-walled. In the second collection, consisting of young seedlings, haustoria were more numerous and were thin-walled and filamentous. Oogonia seen in May. Leach (1931) reports that the mycelium is perennial in the root stock of sugar beet.

2. *Beta maritima*. From Portishead, Som., 1949 and 1952; Berrow, Som., 1953. A few haustoria, which were considerably thickened, were in the leaves. Hyphae were found in the petiole and stem throughout the year.

Geraniaceae

Peronospora conglomerata Fuckel

On *Geranium molle*. From Bristol, 29 April 1952; Windsor, Berks, 18 May 1954. The Bristol specimen was seriously diseased but there were only a few haustoria present. They were filamentous and coiled on themselves. In the Windsor specimen there were many more hyphae but only a very few haustoria. Oogonia were present in May.

The cell walls of the host were noticeably thickened in the region where the hyphae occurred.

Leguminosae

In this family haustoria are not always present. When they do occur they are all simple, hyphal, usually unbranched filaments, which are often covered with a layer of callose.

Peronospora trifoliorum de Bary (*P. aestivalis* Sydow)

On *Medicago* (a forage crop). From Portishead, Som., May, June and August. The extensive development of the hyphae throughout the mesophyll had the effect of separating the host cells. There were only a very few haustoria in small disintegrating areas of the leaves. No haustoria were seen in August material.

Peronospora trifoliorum de Bary (*P. trifoli-repentis* Sydow)

On *Trifolium repens*. (The conidia were 30–35 μ as against 21 μ.) Several collections from Long Ashton, Som., May. Sturdy haustoria, usually with a thickened base, were found in the leaves.

Peronospora trifoliorum de Bary (very variable conidia)

On *Trifolium pratense*. From Hambrook, Glos., May; Wotton-under-Edge, Glos., October. Several collections were made, there were very few hyphae and no haustoria observed. Oogonia were present in May.

Peronospora viciae (Berk.) Casp. (*P. fulva* Sydow)

On *Lathyrus pratensis*. From Bristol, May. A severe infection in which only a few haustoria were found in the leaves and stem. Oogonia were present in the young leaves.

Peronospora viciae (Berk.) Casp. (*P. pisi* Sydow)

On *Pisum sativum*. Infected pods were purchased in Bristol on two occasions in August. Many thin-walled hyphae were present, usually several in one cell (Fig. 3B). Hyphae had penetrated the inner sclerenchymatous layer of the pericarp. Oogonia were present among the hairs which grow on the inner surface of the fruit wall. Similar digitate haustoria are reported by Campbell (1935). The formation of hairs by proliferation of the cells of the pericarp and the development of oogonia among them is reported by Snyder (1934).

P. viciae (Berk.) Casp. (*P. sepium* Gäum.)

On *Vicia sepium*. From Bristol, Slough, Bucks, and Berrow, Som., 1952 and 1953. The conidia of the Berrow material were smaller than those from other places. There were always a few simply branched haustoria. In the early part of the season they were much thickened (Fig. 3A, A1), with a sturdy sheath as a collar at the point of entry. Later in the year, when the host cells, pushed apart by the invading hyphae, were disintegrating, the haustoria were filiform coils filling the cells. The coralloid structure of the mycelium under the lower epidermis was observed during the drought of March 1953.

P. viciae (Berk.) Casp. (*P. viciae* (Berk.) Gäum.)

On 1. *Vicia hirsuta*. From Dorking, Surrey, 4 June 1953. There were no haustoria, the hyphae were becoming autolysed. Oogonia were present in the leaves.

2. *Vicia sativa*. From Wraxall, Som., 30 June 1953; Windsor, Berks, 18 May 1954. There were a few haustoria in the neighbourhood of a vein, the hyphae were becoming autolysed.

Peronospora manshurica (Naoumoff) Sydow

On *Glycine soya*. Hildebrand & Koch (1951) describe and figure haustoria which are 'whip-like'.

ROSACEAE

Only after many macerations had been made were a few filiform haustoria observed with certainty in the two members of this family examined.

Peronospora potentillae de Bary

On *Potentilla sterilis*. From Wotton-under-Edge, Glos., May and June 1954. Haustoria, which were very small, were discovered only after drastic maceration treatment. The hyphae gave a callose reaction with resorcin blue. The mesophyll consisted of very closely packed cells and was permeated by narrow hyphae in very close contact with the host cells.

Peronospora sparsa Berk.

On *Rosa* cv. Tawny Gold. From St Buryan, Cornwall, November 1954. Two collections. The hyphae were becoming autolysed. A few filiform haustoria were found in only one leaf in the epidermal cells, which are the last in the leaf to collapse and die. Oogonia were present in the leaves.

EUPHORBIACEAE

Peronospora euphorbiae Fuckel

On *Euphorbia paralias*. From Berrow, Som., May and June 1954. The stomata of the host are on the upper (adaxial) surface of the leaves, which curl upwards thus sheltering the conidiophores on the upper surface. There were haustoria in some of the mesophyll cells, they were filiform and branched. Oogonia were present in the centre of the leaves.

POLYGONACEAE

Peronospora rumicis Corda

On *Rumex acetosa*. From Wotton-under-Edge, Glos., 30 May 1953. The fungus was present in all the younger leaves, they were swollen and rolled. A few simple hyphal haustoria, some of them mere peg-like protrusions, were enclosed in sheaths of callose.

URTICACEAE

Peronospora debaryi Salmon & Ware

On *Urtica urens*. From Slough, Bucks, 4 November 1952; Lincoln, 19 June 1953; Hambrook, Glos., 23 May 1954. All specimens were only slightly infected and there was no disorganization of the tissues. Only a few haustoria were seen, they were hyphal and sometimes branched and covered with sheaths of callose (Fig. 3C). Oogonia were present in May and November.

Salmon & Ware (1929) describe the haustoria as vermiform, either simple or bifurcate. They figure haustoria which are more delicate in

appearance than those recorded here. These authors were describing plants systemically infected, and their haustoria may have been in the stem and have met with less host resistance.

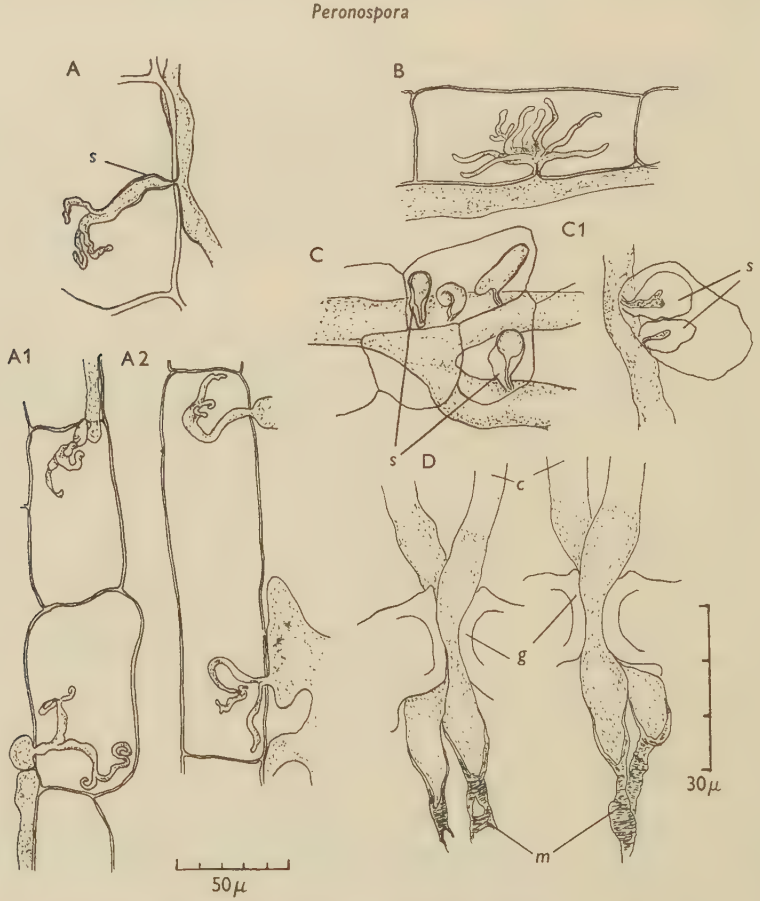

Fig. 4. A, *Peronospora chlorae* in root cortex of *Blackstonia perfoliata*; (*s*) sheath. A 1, A 2, *P. chlorae* in leaf of *B. perfoliata*. B, *P. grisea* in stem of *Veronica persica*. C, C 1, *P. lamii* in leaf of *Lamium purpureum*; (*s*) sheath. D, *P. ficariae* in leaf of *Anemone coronaria*; (*c*) conidiophores, (*g*) guard cells, (*m*) hyphae plugged with callose.

GENTIANACEAE

Peronospora chlorae de Bary

On *Blackstonia perfoliata*. From Cleeve, Wraxall and Portishead, Som., March to November. Conidia were not often evident on plants in the field, but they were developed when small pale plants were placed under a bell-jar indoors. Older flowering plants did not show this phenomenon. Branched hyphal haustoria were found in the leaves, the stem and the roots of infected plants (Fig. 4A, A 1, A 2). Some haustoria were thickened

at the base, but there was not much host resistance. The vertical cell-walls of the stem were thick and collenchymatous, and there were relatively enormous pits through which the haustoria could enter very easily. Oogonia were seen in November.

P. chlorae on *Erythrea centaureum* was shown by Mangin (1895) to have similar stalked and branched haustoria.

Boraginaceae

Peronospora myosotidis is figured by Mangin (1895). Haustoria are hyphal and branched, very much thickened at the point of entry into the host cell.

Solanaceae

Peronospora tabacini Adam.

On *Nicotiana tabacum*. Haustoria, which are hyphal and coiled, are figured by Angell & Hill (1932).

Scrophulariaceae

Downy mildews infecting members of this family all had haustoria which were hyphal, often branched and with a stalk of varying proportions. The development of the sheath varied and was not a specific character.

Peronospora grisea Unger. (*P. agrestis* Gäum.)

On *Veronica agrestis*. From Bristol; Wotton-under-Edge, Glos., April to October. Small numbers of hyphal digitate haustoria were always found in the leaves. Oogonia were seen in April and May.

Peronospora grisea Unger.

On 1. *Veronica beccabunga*. From Wotton-under-Edge, Glos.; Portbury, Som. The infection is often systemic and evident as soon as the shoots begin to grow in spring.

In the leaf, which is loosely constructed, the hyphae are wide and branched, in the stem they are narrow and run from node to node with few branches. Haustoria were present in the leaf and stem throughout the year in the systemically infected plants. A leaf of a newly infected plant was found to lack haustoria. Haustoria were most elaborate in the stem, towards the end of the season hyphae running through the cortex of the stem produce haustoria which enter almost every cell with which they are in contact.

2. *Veronica persica*. From Wraxall, Som., 30 June and 7 July 1953. Digitate haustoria were present in the leaves in June (Fig. 4B). There were no haustoria in the specimens collected in July. Oogonia were present in July.

3. *Veronica officinalis*. Digitate haustoria were recorded by Wells (unpublished B.Sc. thesis, Bristol University 1949).

4. *Veronica serpyllifolia* (*P. verna* Gäum.). From Bristol, 1 May 1952 and 14 April 1953 (seedlings); Pensford, Som., April, May 1954. Haustoria, which were scarce, were seen in all specimens in both stem and leaf.

Peronospora sordida Berk. & Broome

On *Scrophularia nodosa*. From Cleeve, Som., 13 May 1954. One very young specimen. Hyphae stained with resorcin blue gave a callose reaction. They grew throughout the leaves. There were many haustoria in the leaves, stalked and branched and surrounded by sheaths which varied in form. In some instances the sheath was diffuse and jelly-like, enclosing the tips of the haustoria in a thin bright blue cloud when it was stained with resorcin.

Labiatae

Peronospora lamii A. Braun

On 1. *Lamium purpureum*. From Frampton-on-Severn, Glos., April 1954, July 1953; Slough, Bucks., August. The July and August specimens were newly infected, the other was a systemic infection. Haustoria, which were club-shaped, were present in the leaf, petiole and stem. In slightly infected plants there was evidence of great host resistance in the form of thickened sheaths around the haustoria, particularly at their free ends. In the more seriously infected host plants the haustoria were much more numerous with several of different sizes in a cell (Fig. 4C, C1). In several instances the sheath could be seen to be a gel and could be moved by a tap on the coverslip. Oogonia were present in the leaves in April and August.

2. *Stachys sylvatica*. Similar club-shaped haustoria were observed by Wells (unpublished B.Sc. thesis, Bristol University, 1949).

Plantaginaceae

Peronospora alta Fuckel

On *Plantago major*. From Bristol district, May to September. There were few haustoria, they were hyphal, sometimes covered with a sheath when they were coiled on themselves. The smaller haustoria were thickened all over their surface. Oogonia were present in May.

Rubiaceae

The haustoria of species of *Peronospora*, which are parasitic on members of this family, were all hyphal, branched and more or less stalked. The chief variations were caused by differences in the development of the haustorial sheath.

Peronospora calotheca de Bary

On 1. *Asperula odorata*. From Bristol district, April and May. Hyphae grew throughout the plants. Haustoria were stalked and branched when they were most fully developed. The most elaborately branched haustoria occurred in the cortex of the stem (Fig. 3D). Oogonia were present in the stem in May.

2. *Galium aparine* (*P. aparines* (de Bary) Gäum.). From Bristol, April, May and June; Slough, Bucks, March and April; Long Ashton and Portishead, Som., April.

There were haustoria in leaf and stem in all collections, most fully developed in the stem. In 1952 the haustoria were thin-walled, in March 1953, after a long period of drought, they were much thickened and masses of 'coralloid' hyphae were accumulated under the lower epidermis of the leaves. These hyphae gave rise to conidiophores and conidia as soon as the plants were placed in a moist chamber. Oogonia were present in these plants in quantity. In all collections, oogonia were found in March and June, the beginning and end of the season.

Peronospora calotheca var. *sherardiae* (de Bary) Rabenh.

On *Sherardia arvensis*. From Wraxall, Som., October and November 1953. In October there were haustoria in the stem, particularly in the region of the endodermis. In the second collection there were haustoria in the leaves which by then were more generally chlorotic. There were thick sheaths on the haustoria. The cells of the host were collenchymatous.

Dipsacaceae

Peronospora dipsaci Tul.

On *Dipsacus sylvestris* was recorded by Christy (unpublished B.Sc. thesis, Bristol University, 1950) as having hyphal haustoria, often stalked and branched in leaf and petiole.

MONOCOTYLEDONS

Liliaceae

Peronospora destructor (Berk.) Casp.

On *Allium ascolonicum*. From Aberdeen, Scotland, 26 May 1952. The leaves were pale and collapsed. Hyphae ran throughout the length of the leaves, most of the cells contained simple or forked hyphal haustoria. In many instances more than one haustorium was present in the elongated cells of the leaf bases. There was no thickening at the point of entry, immediately after entering the cell through a narrow hole in the wall the haustoria widened and continued as a tube of even bore which was sometimes branched. There were no callose sheaths covering the haustoria. Similar haustoria have been described by Murphy & M'Kay (1926) and Yarwood (1943).

(*b*) *Haustoria of the genus* Pseudoperonospora *Rostowzew*

Pseudoperonospora humuli (Myabe & Tak.) G. W. Wilson

On *Humulus lupulus*. From Bristol (preserved); Normandy, August 1952; Wotton-under-Edge, Glos., October 1953. A few haustoria were usually found in the stem and petiole and appeared to consist of small solid masses covered with callose. The Wotton specimens were from a hedge which had been pruned and the Hop plant was growing vigorously,

although the young shoots were diseased. In the stem there were haustoria which consisted of a thick basal portion from which thinner walled branches, vermiform and coiled extensions, had grown (Fig. 5 C, C 1).

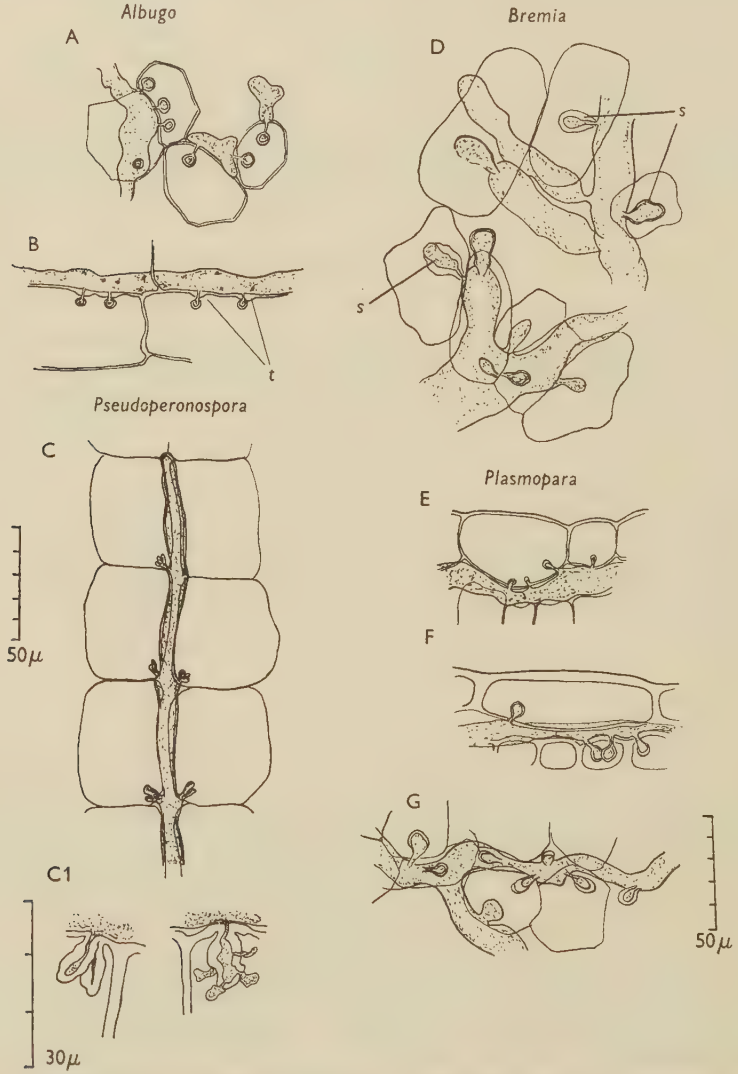

Fig. 5. A, *Albugo candida* in *Alyssum saxatile* leaf. B, *Albugo tragopogonis* in *Tragopogon minor* leaf; (*t*) thickening of host cell-wall. C, *Pseudoperonospora humuli* in *Humulus lupulus* pith of stem. C 1, the same under oil immersion. D, *Bremia lactucae* in *Lactuca sativa*; (*s*) sheath of haustorium. E, *Plasmopara nivea* in *Anthriscus sylvestris* leaf, very small haustoria. F, *P. pygmaea* in *Anemone nemorosa* leaf. G, *P. pusilla* in *Geranium pratense* leaf.

Millasseau (1928) reported haustoria of two types, small and knob-like in the leaves and stalked and digitate in the stem. They are here interpreted as fundamentally hyphal but usually underdeveloped owing to the thick sheath of callose produced by the host cells.

Pseudoperonospora cubensis (B. & C.) Rostowzew

On cucumber, is described by Clinton (1904) as having haustoria which are short and ovate but sometimes found to develop finger-like processes from their swollen tips. They are similar therefore to those of *P. humuli* above.

Pseudoperonospora urticae (Lib.) Salm. & Ware

On *Urtica dioica*. From Ashurst, Hants, September; Dorking, Surrey, October. The infections were slight. Few hyphae were discernible, there were no haustoria. The conidiophores, which arose singly through the stomata, were sealed off just inside the leaf. This condition was similar to that occurring in *Humulus lupulus* when old leaves are freshly infected with mildew at the end of the year. The hyphae were short-lived and became autolysed as soon as conidia were formed. (See also *Anemone coronaria*, p. 86).

(*c*) *Haustoria of the genus* Bremia *Regel*

Bremia lactucae Regel

On 1. *Senecio vulgaris*. From Bristol district throughout the year.
2. *Lactuca sativa*. From Bristol, 11 January 1954.
3. *Sonchus oleraceus*. From Bristol, May 1952.
4. *Tragopogon minor*. From Bristol, June 1953.

The hyphae were irregular in form, often wide, filling large intercellular spaces, at other times being very narrow where the fungus grew between closely packed cells. Haustoria were produced and entered the host cells in all directions. There were usually several haustoria in a single cell. The haustoria were essentially flask-shaped vesicles, though they varied in size and thickening, some of the larger ones were asymmetrical (Fig. 5D). Each haustorium was enclosed in a sheath of callose which varied in thickness and in distribution over the surface of the haustorium. Any differences seen in the various host plants examined could be related to the type of cell in which they were growing, thus the haustoria in the large parenchymatous cells of the pith of a stem of *Sonchus oleraceus* were larger and more clavate than those in the thicker-walled cells of the cortex of the stem of *Senecio vulgaris*.

(*d*) *Haustoria of the genus* Plasmopara *Schroeter*

All species of *Plasmopara* examined were found to have large numbers of haustoria which were flask-shaped vesicles, usually with a wide stalk and entry channel. In any one specimen there was considerable range of size and differences in the development of the callose sheath.

1. *Plasmopara viticola* (Berk. & Curt.) Berlese & de Toni on cultivated *Vitis vinifera*. From Normandy, September 1952. Mangin (1895) figures similar small haustoria in the leaf.

2. *Plasmopara pusilla* (de Bary) Schroet. on *Geranium pratense*. From two places near Wotton-under-Edge, Glos., May and July (Fig. 5E).

3. *Plasmopara pygmaea* (Unger) Schroet. on *Anemone nemorosa*. From Blaise, Bristol, April 1954; Cleeve, Som., May 1952 (Fig. 5F).
4. *Plasmopara nivea* (Unger) Schroet. on *Aegopodium podagraria*. From Bristol, May; Henbury, Glos., April 1954. de Bary (1863) figures similar haustoria for this fungus.
5. *Plasmopara nivea* (Unger) Schroet. on *Anthriscus sylvestris*. From Wotton-under-Edge, Glos., April and May 1953. The haustoria were extremely small (Fig. 5G). Mangin (1895) figures similar haustoria on 'Cerfeuil'.
6. *Plasmopara densa* (Rabenh.) Schroet. on *Odontites verna*. From Wraxall, Som., June, had many haustoria of the usual *Plasmopara* type. Mangin (1895) figures similar haustoria on *Euphrasia odontites* (i.e. *Odontites verna*.)
7. *Plasmopara densa* (Rabenh.) Schroet. on *Rhinanthus minor*. From various places near Bristol, June 1952–4. The haustoria were more varied in size and shape than those of the same fungus on *Odontites*, many were asymmetrical. Oogonia were present on 3 June 1954.
8. *Plasmopara epilobii* (Rabenh.) Schroet. on *Epilobium parviflorum*. From Ashurst, Hants, September 1954. de Bary (1863) figures similar haustoria for *P. densa* on *Rhinanthus minor*.

(*e*) *The Haustoria of the genus* Sclerospora *Schroeter*

Sclerospora philippinensis Weston on Maize is reported by Weston (1920) to develop haustoria in the mesophyll which are 'simple papillate to tubular and somewhat lobed'. He quotes Rutgers's description of haustoria of *S. javanica* Palm. as being digitate. Both these fungi therefore have hyphal haustoria.

(*f*) *The haustoria of the genus* Albugo Pers. ex S. F. Gray

All haustoria formed by members of this group were small globose vesicles with a narrow stalk at the point of entry into the host cell. There were usually several in a single cell. Callose sheaths of varying thickness were present. The perforation channel through the host cell-wall was always narrow and at right angles to the wall which was often thickened at this point. Some haustoria contained dense protoplasm and stained deeply, others remained clear. According to Wager (1896) the haustoria stain deeply when they are first formed, later, they contain only a watery fluid.

Albugo candida (Pers.) Kuntze

On 1. *Cardamine hirsuta*. From Bristol, June.
2. *Sisymbrium officinale*. From Cleeve, Som., August 1952.
3. *Alyssum saxatile*. From Bristol, October 1953, 1954.
4. *Aubretia*. From Hambrook, Glos., August 1954.
5. *Arabis*. From Holford, Som., September 1954.
6. *Capsella bursa-pastoris*. From Bristol, throughout the year.

Albugo tragopogonis (DC.) S. F. Gray

On *Tragopogon minor*. From Bristol, July. The haustoria were in all essentials the same as those of *A. candida*. The thickening of the host cell wall at the point of entry of the haustoria was very pronounced (Fig. 5B).

RELATION BETWEEN HOST AND PARASITE

Although there is no obvious chemical alteration of the host cell-walls, those of some leaves become hard and brittle perhaps as a result of the continual removal of nutriment. Distortion of the host plant, the curling of the leaves of *Veronica beccabunga*, *Anemone coronaria*, *Papaver* sp., *Rumex acetosa* and *Galium aparine*, for example, could be brought about by the drying out and shrivelling of the lower surface of the leaf.

The volume of the mycelium may bring about distortion and disruption of the host and add to the virulence of the disease. The development of the 'coralloid' structures seen in many species under certain weather conditions causes considerable distortion. Here the rapid increase in volume of the hyphae, as they grow under the lower epidermis towards the stomata, has the effect of raising the epidermis and completely disrupting the leaf.

Again, destruction of the host is rapidly effected when the stomata are blocked as the conidiophores develop. When this occurs photosynthesis must be curtailed immediately and the transpiration stream seriously interrupted. The extent and importance of this effect varies with the habit of the parasite; those species where several conidiophores grow through a single stoma will be the most virulent parasites. In the hop and the vine, both climbing plants, the leaves become shrivelled and brittle when their stomata are blocked in this way.

The nature of the host tissue through which the hyphae grow influences the development of haustoria. They are not apparently essential in a close-celled and turgid leaf until the host tissues are disrupted by the bulky mycelium. When the leaf is in a state of collapse and the mesophyll cells, separated from each other, are practically moribund, a few narrow hyphal haustoria develop. This is the type of growth found in *Ranunculus repens*, *R. bulbosus*, *Trifolium pratense* and *Potentilla sterilis*, and others. Haustoria were not formed in several species when the infection was new and on young plants. Sufficient nutriment is probably obtained for some time through the hyphal walls when these are in close contact with the host. *Stellaria media*, *Veronica beccabunga* and *Ranunculus ficaria* are good examples of hosts where this condition obtains.

If the growth of a hypha is hindered as it passes between the host cells, haustoria develop where the obstruction occurs, for example at the corners of the elongated cells of the cortex of pith in *Humulus lupulus* and in *Papaver* sp.

Haustoria which develop in leaves, where presumably the food supply is good, are smaller and less elaborate than those found in the fully grown cells of the stem containing less nutriment. The shape of the haustoria is

within limits dependent upon the ease with which food is to be obtained. In slight infections of healthy tissues where food is plentiful the haustoria are simple, whereas, when the host is seriously affected and food supply is becoming critical, the haustoria show elaborate coiling and branching, thus presenting a large absorbing surface.

No correlation was to be observed, however, between the severity with which the host was attacked by any particular mildew and the form of the haustoria. The most destructive of all those studied here was *Peronospora destructor* where the haustoria are mainly of a simple hyphal or filamentous type.

Further, no correlation was discovered between the family to which the host plant belonged and the form assumed by the haustoria of the invading parasites. Numerically there are on the whole more mildews parasitic on members of those families commonly considered to be most primitive, but there are important exceptions, such as the families Scrophulariaceae and Rubiaceae.

DISCUSSION

The morphology of members of the Peronosporales is very much determined by local environment. On the whole, this study of the form and structure of hyphae and haustoria does not offer help in classification (see Table 3).

Table 3. *Types of haustoria*

Family	Genus	Type
Albuginaceae	*Albugo*	Regular knobs or spheres
	Basidiophora	Regular knobs or spheres
	Sclerospora philippinensis	Hyphal, lobed
	S. javanica	Hyphal, digitate
Peronosporaceae	*Plasmopara*	Small irregular vesicles
	Pseudoperonospora	Hyphal, digitate
	Bremia	Small irregular vesicles
	Peronospora, not *P. parasitica*	Hyphal, often branched
	P. parasitica	Large lobed vesicles

The haustoria of all species of *Peronospora*, except those on Cruciferous hosts, are basically hyphal and filamentous. The lobed vesicular haustoria on Crucifers are characteristic (the only exception is that on *Coronopus lepidii*) and are not paralleled in any other group. Within the species group *Peronospora parasitica* there is perhaps some indication of variations of value, though they may be related to the nature of the host leaf and be primarily more of a host-parasite relation result than a true function of the parasite itself. Large bilobed and thin-walled haustoria are typical of mildew found on garden wallflower plants; regular vesicles, not lobed, are characteristic of the mildew on Brompton stock. Gäumann (1918*a*), by means of measurements of conidiophores and conidia and by cross-inoculation methods, has subdivided *P. parasitica* into no less than fifty-two separate groups; these are probably different strains of the one species.

A series of stages leading towards the obligate parasitism of the higher types within the group Peronosporales may possibly be made out by a

consideration of the morphology of the mycelium taken together with the method of reproduction, in the more important genera. There is a wide host range among the simpler species of *Phytophthora* and the hyphae are intracellular. The more advanced species, *P. infestans*, is limited to a more narrow host range. Here, haustoria of a simple filiform type have been described (Blackwell, 1953) in strains of the fungus, limited to certain varieties of the host plant (Potato cv. Majestic). *P. infestans* is a species difficult to grow in culture, whereas the simpler members of the genus offer less difficulty in this respect.

Albugo, together with the lower members of the Peronosporaceae, *Basidiophora* and *Plasmopara*, have very numerous small regular and vesicular haustoria; they are typical and successful obligate parasites which, for a long time after infection, do not seriously damage their hosts.

Bremia, which on the grounds of the method of germination of its conidia and on the morphology of the conidiophores, is usually considered to be at the same level of advancement as *Peronospora*, has numerous relatively simple vesicular haustoria. These are intermediate in size and form between those of *Plasmopara* and *Peronospora*. This fungus does little damage to its host and may be regarded as having achieved a balanced relationship comparable with the state of affairs in the rust fungi.

Peronospora, other than *P. parasitica*, has haustoria which show a secondary proliferation and a return to a hyphal form. The mycelium may be considered to be partially intracellular but with a morphological difference between the inter and intracellular parts of the mycelium. This return to an almost intracellular habit is often associated with an increasing amount of disruption and consequent destruction of the host. However, in at least one example, despite the elaboration of the hyphal haustoria, e.g. *P. chlorae* on *Blackstonia perfoliata*, a balanced relationship, almost mycorrhizal in nature, is achieved. This fungus which reaches all parts of the host, including the root and the xylem of the vascular bundles, lives in its host throughout the year, producing conidia only when external conditions are particularly suitable for the purpose.

The Peronosporales as a whole do not show the perfectly balanced relationship between host and fungus exhibited by the rust fungi, and this may perhaps be related to the clumsy nature of their somatic structure. The coenocytic mycelium, consisting of hyphae which can vary in shape and size and which absorb nutriment all over their surface, fills every available intercellular space in the host tissue. This enveloping mass of mycelium interferes too much with the life processes of the host plant and results in the rapid destruction of both host and parasite.

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REFERENCES

Angell, H. R. & Hill, A. V. (1932). Downy mildew (Blue mould) of Tobacco in Australia. *Bull. Coun. sci. industr. Res. Aust.* 65.

Arens, K. (1929). Untersuchungen über *Pseudoperonospora humuli* (Miyabe u. Takah.), den Erreger der neuen Hopfenkrankheit. *Phytopath. Z.* **2**, 169–193. *Rev. appl. Mycol.* **9**, 59).

Arx, J. A. von & Noordam, D. (1951). Valse meeldauw (*Peronospora pulveracea* Fuckel) op *Helleborus niger*. *Tijdschr. PlZiekt.* **57**, 32–34.

de Bary, A. (1863). Récherches sur le développment de quelques champignons parasites. *Ann. Sci. nat., Bot.* (Ser. 4), **20**, 1–148.

de Bary, A. (1887). *Comparative morphology and biology of the fungi*. Oxford.

Blackwell, E. M. (1953). Haustoria of *Phytophthora infestans* and some other species. *Trans. Brit. mycol. Soc.* **36**, 138–158.

Campbell, L. (1935). Downy mildew of peas caused by *Peronospora pisi* (de Bary) Syd. *Bull. Wash. St. agric. Exp. Sta.* p. 318.

Clinton, G. P. (1904). Downy mildew of musk melon and cucumbers. *Rep. Conn. agric. Exp. Sta.* pp. 329–362.

Eschrich, W. (1954). Ein Beitrag zur Kenntnis der Kallose. *Planta*, **44**, 532–42.

Fischer, A. (1892). Phycomycetes. *Rabenhorst Krypt. Flor.* Abt. **4**, 442.

Foster, J. W. (1949). *Chemical activities of fungi*, p. 97. New York.

Fraymouth, J. & Hawker, L. E. (1952). The use of commercial 'Pectozyme' in the anatomical study of parasitic fungi within plant tissues. *Trans. Brit. mycol. Soc.* **35**, 91.

Gäumann, E. (1918*a*). Ueber die Formen der *Peronospora parasitica* (Pers.) Fries. *Beih. bot. Zbl.*, 1 Abt., **35**, 1–143, 395–533.

Gäumann, E. (1923). Beiträge zu einer Monographie der Gattung *Peronospora* Corda. *Beitr. Kryptogamenfl. Schweiz*, **5**.

Hildebrand, A. A. & Koch, L. W. (1951). A study of systemic infection by downy mildew of soybean with special reference to symptomatology, economic significance and control. *Sci. Agric.* **31**, 505–18.

Ikata, S. & Yamauti, K. (1941). Notes on the haustoria of some species of *Peronospora*. *Ann. phytopath. Soc. Japan*, **10**, 326–328.

Klebahn, H. (1925). Über das myzel der *P. pulveracea* Fuckel. *Z. Pflkrankh.* **35**, 15–69.

Leach, L. D. (1931). Downy mildew of the beet caused by *P. schachtii* Fuckel. *Hilgardia*, **7**, 203–251.

Magnus, P. (1894). Das auftreten der *P. parasitica* beeinflusst von der Beschaffenheit und dem Entwickelungszustande der Wirthspflanze. *Ber. dtsch. bot. Ges.* **12**, 39.

Mangin, L. (1895). Récherches anatomiques sur les Péronosporées. *Bull. Soc. Hist. nat. d'Autun*, **8**, 55–108.

Mangin, L. (1910). Nouvelle observations sur la callose. *C.R. Acad. Sci., Paris*, **151**, 279–283.

Millasseau, J. (1928). Contribution a l'étude morphologique du *Peronoplasmopara humuli* Mybe et Tak. *Ann. Épiphyt.* **14**, 175–198.

Murphy, P. A. & M'Kay, R. (1926). The downy mildew of onions (*P. schleideni*) with particular reference to the hibernation of the parasite. *Sci. Proc. R. Dublin Soc.* **18**, N.S., 17–28, 237–261.

Rice, M. A. (1927). The haustoria of certain rusts and the relation between host and pathogene. *Bull. Torrey bot. Cl.* **54**, 63–153.

Richards, M. C. (1939). Downy mildew of Spinach and its control. *Bull. Cornell. agric. Exp. Sta*, no. 718.

Salmon, E. S. & Ware, W. M. (1929). Two downy mildews of the nettle. *Pseudoperonospora urticiae* (Lib.) Salmon et Ware and *Peronospora debaryi* nomen novum. *Trans. Brit. mycol. Soc.* **14**, 38–60.

Snyder, W. C. (1934). *P. viciae* and internal proliferation in Pea pods. *Phytopathology* **24**, 1358–1365.

Thatcher, F. S. (1939). Osmotic and permeability relations in the nutrition of fungus parasites. *Amer. J. Bot.* **26**, 449–458.

Thomas, R. C. (1928). Composition of fungus hyphae 1. The Fusaria. *Amer. J. Bot.* **15**, 537–547.
Thung, T. H. (1926). Opmerkingen over *P. parasitica* op Kool (Bemerkungen über die Kohl-peronospora) *Tijdschr. PlZiekt.* **32**, 161–179.
Wager, H. (1896). On the structure and reproduction of *Cystopus candidus*. *Ann. Bot., Lond.*, **10**, 295–342.
Ware, W. M. (1926). *Pseudoperonospora humuli* and its mycelial invasion of the host plant. *Trans. Brit. mycol. Soc.* **11**, 91–107.
Weston, W. H. Jr. (1920). Phillipine downy mildew of Maize. *J. agric. Res.* **19**, 97–122.
Yarwood, C. E. (1943). Onion downy mildew. *Hilgardia*, **14**, 595–691.
Yossifovitch, M. (1929). *Peronospora arborescens* (Berk.) de Bary, parasite très important de *Papaver somniferum* en Yougoslavie. *Rev. Path. vég.* **16**, 235–270.

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STREAM SPORA IN NIGERIA

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(With 1 Text-figure)

An illustrated account is given of spores found in the scum and foam of a stream near Ibadan, Nigeria. Spores belonging to *Actinospora megalospora* Ing., *Triscelophorus monosporus* Ing. and *Campylospora chaetocladia* Ranzoni were recognized. Other types of spore seen appear to belong to undescribed species of aquatic Hyphomycetes.

During a brief visit to University College, Ibadan, Nigeria, at the end of May 1955, I had the opportunity of examining the scum and surface foam from a stream (the River Awba) flowing through secondary bush, dominated by oil and raffia palms with bamboo undergrowth, in the grounds of the Botany Department. The scum and foam, as in streams in Britain (Ingold, 1942) contained a rich 'spora' presumably composed largely of the spores of aquatic Hyphomycetes. Just as in Britain this 'spora' is dominated by tetra-radiate spores with long arms, and in addition there are many spores which are long and threadlike and these tend to be sigmoid or comma-shaped with the curvature lying in more than one plane. It should be noted that the spores do not get into the scum or foam because they float. Indeed, they are slightly denser than water. The scum or foam acts as a spore trap in which spores which happen to be brought to the surface get caught.

Only a few of the spores encountered could be referred with confidence to described species. These were *Actinospora megalospora* Ing. (Fig. 1 A), *Triscelophorus monosporus* Ing. (Fig. 1 B) and *Campylospora chaetocladia* Ranzoni (Fig. 1 C). A score or more spores of the first species were seen and hundreds of the other two. *C. chaetocladia* was described by Ranzoni (1953) from California. There is complete agreement between his description of the remarkable spores of this aquatic Hyphomycete and the Nigerian specimens. A spore clearly belonging to the same genus was figured by Ingold & Ellis (1952) from a sample of scum collected at Wheatfen in Norfolk, and indeed many of these spores were seen and drawn. Although the *Campylospora* spores from Wheatfen have the same general form and are of much the same size as in the type species, their appendages are distinctly shorter and a second species may, perhaps, be involved.

Spores of a species of *Tricladium* (Fig. 1 D) were abundant, but they did not seem to agree exactly with those of any of the described species.

The striking spores shown in Fig. 1 E may belong to a species of *Clavariopsis*, and perhaps the very different spores illustrated in Fig. 1 F might also find a place in the same genus. In *Clavariopsis* a relatively stout, club-

shaped, first-formed arm is produced and the three other arms arise simultaneously as outgrowths from its apex.

The large tetra-radiate spores seen in Fig. 1 G were quite common and very uniform. It seems likely that these may belong to a new species of *Actinospora*. Many of the large multi-radiate spores shown in Fig. 1 H were

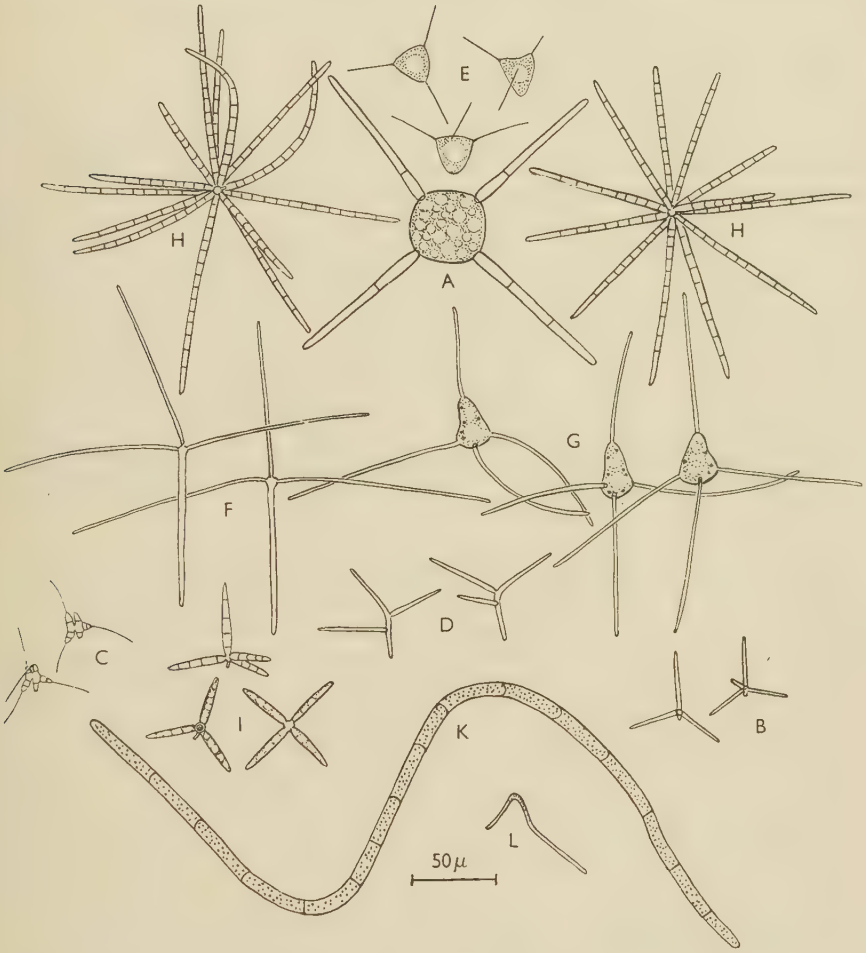

Fig. 1. Spores from Nigerian stream all drawn to the same scale from living specimens with the aid of a camera lucida.

observed. In this spore-type there is a small central spherical part to which all the arms are attached. It seems almost certain that these spores belong to an undescribed genus.

Spores of the type shown in Fig. 1 I were very frequent. Here the four arms, which are relatively stout, diverge from a small spherical, central region which has a short projection no doubt concerned with attachment to the parent conidiophore. I have frequently seen spores very much like

these (Ingold, 1942, fig. 1, 17), but of a larger size and with more tapering arms, in the 'spora' of some streams in Britain, but I have never seen them attached.

About half a dozen types of long thread-like spores of more or less sigmoid form were seen in the scum and foam, but I have made little attempt to record them. One common type was exceedingly large (Fig. 1 K), but most were much smaller (e.g. Fig. 1 L). Generic identification based on this type of spore is almost impossible.

To sum up, the following points may be made: (1) that spores of aquatic Hyphomycetes seem to contribute largely to the stream 'spora' in Nigeria as in Britain; (2) that some of the species found at Ibadan have also been found in Britain, but these appear to be species which are rather rare in Britain; (3) that most of the types of spore in the Ibadan stream belong to undescribed species; and (4) that those species which are exceedingly common in Britain (especially *Tetracladium marchalianum* de Wild., *Clavariopris aquatica* de Wild., *Lemonniera aquatica* de Wild. and *Alatospora acuminata* Ing.) are not apparently represented in the Ibadan 'spora'.

REFERENCES

Ingold, C. T. (1942). Aquatic Hyphomycetes of decaying alder leaves. *Trans. Brit. mycol. Soc.* **25**, 339–417.

Ingold, C. T. & Ellis, E. A. (1952). On some Hyphomycete spores, including those of *Tetracladium maxilliformis*, from Wheatfen. *Trans. Brit. mycol. Soc.* **35**, 158–161.

Ranzoni, F. V. (1953). The aquatic Hyphomycetes of California. *Farlowia*, **4**, 353–398.

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SOME NEW SPECIES OF SOIL MOULDS

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(With Plate 3)

Aspergillus funiculosus G. Smith, from Nigerian soil; *Penicillium atrovenetum* G. Smith, type strain from soil, Sussex Downs; and *P. canadense* G. Smith, from soil in Douglas fir nursery, Vancouver, are described as new to science.

Aspergillus funiculosus sp.nov. (Pl. 3, fig. 1)

Coloniae in agaro Czapekii celeriter crescentes, virido-flavae dein lente subolivaceae, funiculosae, reverso primum irregulariter olivaceo purpureoque dein subzonato, sordido-flavo, rubro, purpureoque, denique brunneo-purpureo; capitula conidica primum globosa, dein radiata, ad 140 μ diam.; conidiophora hyphis aetheriis oriunda, brevia, glabra, 4–7 μ diam., angustiora sub vesicula; vesiculae globosae, 10–23 μ diam., in toto fertiles; metulae absentes; phialides 6–7 × 2–2·5 μ; conidia globosa aut subglobosa, echinulata, plerumque 3·8 μ diam. Fungus in solo, Ibadan, Nigeria, inventus.

Colonies on Czapek agar spreading, at first bright greenish yellow, gradually becoming duller from centre outwards, always with yellow edge, forming a fairly thick mat, definitely funiculose; reverse at first dull olive with purplish patches, then broadly zonate in shades of olive to mahogany red or purple, finally deep brownish purple. On other common culture media growth is similar, but the reverse colour tends to vary, even in parallel cultures on the same medium. Conidial heads globose then somewhat loosely radiate, with the smaller ones loose from the beginning, up to 140 μ diam.; stalks mostly arising from aerial hyphae, short, sinuous, smooth, 4–7 μ diam., tapering appreciably just below the vesicle; vesicles globose or nearly so, fertile all over, 10–23 μ diam.; metulae entirely lacking; phialides 6–7 × 2–2·5 μ; conidia thick-walled, coarsely roughened, globose or nearly so, mostly 3·8 μ diam.

Isolated by R. M. Jackson from soil, Ibadan, Nigeria, and sent to the Commonwealth Mycological Institute. Culture received from the C.M.I. in January 1954, as I.M.I. 54,397, and maintained in the L.S.H.T.M. collection as BB 303.

The place of this species in the Thom & Raper classification (1946) is doubtful. The yellow-green colour and tuberculate conidia suggest affinity with the *A. flavus-oryzae* group, but, unlike the species of this group, *A. funiculosus* has stalks which are quite smooth, and conidial heads which are consistently devoid of metulae. There is some resemblance to the *A. versicolor* group, but, again, the lack of metulae denies it a place here. The only group of Aspergilli in which all the species have smooth stalks, heads without metulae, and radiate spore chains is the *A. glaucus* group,

but the colony characteristics of the new species do not suggest affinity with this group, and no sign of perithecium formation has been observed.

Penicillium atrovenetum sp.nov. (Pl. 3, figs. 3, 4)

Coloniae in agaro Czapekii subrestrictae, densae velutinae, coeruleae dein atrovenetae, margine angusto albo, guttulis multiplicis subflavis, reverso flavo-brunneo; penicilli compacti, biverticillati aut asymmetrici; conidiophora laevia aut subasperula, ad 300 μ longa et 2·5–4 μ diam.; metulae 11–12 × 3–4 μ; phialides 8–10 × 2–2·8 μ; conidia globosa, asperula, (2·7) 3–3·6 μ diam.; catenae conidiorum longae, in pluribus columnis laxis aut implicatae. Habitat in solo. Cultus typicus in solo, Sussex Downs, Anglia, inventus.

Colonies on Czapek agar growing somewhat restrictedly, thick velvety, bright bluish green (*venetus*) at first, rapidly turning greyer and becoming deep greyish blue, almost black in age, with narrow white edge during the growing period; respiration drops numerous, faint yellow, drying up and leaving the surface apparently pitted; reverse deep yellowish brown, diffusing very slightly. Colonies on potato dextrose agar very similar; on malt agar somewhat similar but with more rapid and spreading growth. Penicilli biverticillate (but not with the appearance of the Biverticillata-Symmetrica) or asymmetric, with a terminal cluster of metulae, often of unequal length, and usually one branch from a lower node, bearing metulae and phialides or phialides alone (Pl. 3, figs. 3, 4); conidiophores mostly long, up to about 300 μ, arising from the substrate or from a basal felt of hyphae, smooth or slightly rough, 2·5–4 μ diam.; metulae 11–12 × 3–4 μ, often widening somewhat towards the apex; phialides abruptly pointed (not acuminate), 8–10 × 2–2·8 μ; conidia globose, conspicuously rough, exceptionally 2·7 μ but mostly 3–3·6 μ diam.; conidial chains long, in several loose columns, one to each metula, but sometimes becoming tangled.

Type strain, L.S.H.T.M. catalogue no. S.M. 683, isolated from soil from the Sussex downs in 1952. Two other strains are maintained in culture: S.M. 715, found in soil from the sand-dunes, Perranporth, Cornwall, 1952; and a much older isolate, M. 467, from the soil of a mangold field, Norfolk, 1943.

This species is closely related to *P. herquei* Bainier, both morphologically and biochemically. However, it is very doubtful whether anyone attempting to identify a fresh isolate would seek it amongst the Biverticillata-Symmetrica, in which group *P. herquei* is placed by Raper & Thom (1949). In my opinion *P. herquei* is wrongly placed here. Raper & Thom give as their reasons for this placement: (1) 'The penicilli are typically biverticillate and usually symmetrical or nearly so'. However, the phialides are quite different from those which are such a distinguishing feature of the Biverticillata-Symmetrica, and this type of penicillus, more or less symmetrically biverticillate, but with abruptly pointed phialides, is found in the *P. citrinum* series and in the *P. brevi-compactum* series. (2) 'Colonies typically produce varying amounts of yellow encrusted hyphae'. So do *P. multicolor* G., M. & P. and *P. citreo-viride* Biourge, both placed in the Mono-

verticillata. (3) 'Many strains produce a fragrant odour suggesting apples or black walnuts'. Fragrant odours, whilst not common in species of *Penicillium*, are not confined to any one section of the genus.

The penicillus in *P. atrovenetum* is at first more or less symmetrically biverticillate, although even then it is not unusual to find metulae of varying length in the same head (Pl. 3, fig. 3), but later a secondary branch normally develops which makes the whole structure asymmetric (Pl. 3, fig. 4), and, in fact, strongly suggestive of the *P. chrysogenum* series. Such asymmetric penicilli are found also in *P. herquei*, though not so frequently as in *P. atrovenetum*.

P. olsoni Bainier has not been seen in culture, but may be, as Raper & Thom suggest, merely an unusual strain of *P. herquei*.

The only other species of this series, *P. novae-zeelandiae* van Beyma, has penicilli of much the same type as *P. herquei*, roughly symmetrical, but with an occasional branch from below the terminal cluster of metulae, and with abruptly tapering phialides. It is distinguished from all other species of the genus by its production of abundant black sclerotia.

The colonies of the species of this series, including *P. atrovenetum*, are not truly velvety in the sense of the great majority of the conidiophores arising from submerged hyphae, but the general appearance suggests the Velutina, and the texture of the colonies is not unlike that of many isolates of *P. brevi-compactum*, or even *P. chrysogenum*. It is therefore proposed that a better placement of this series is in the Asymmetrica-Velutina.

Penicillium canadense sp.nov. (Pl. 3, fig. 2)

Coloniae in agaro Czapekii late crescentes, tenues, velutinae, primum substramineae dein nubiliores, subzonatae, margine fimbriato, guttulis nullis, reverso incolorato; coloniae in agaro 'malt' dicto celeriter crescentes, subfloccosae, albae dein virido stramineae, denique nubiliores, cum non nihil mycelio aetherio albo; penicilli breves, compacti, plerumque quater ramosi, elementis in ordine angustioribus; conidiophora glabra, ad 600 μ longa, rigida, parte interiore granulosa, 5–5·6 μ diam.; rami 8–12 × 4–5 μ; ramuli 8–10 × 3.5–4 μ; metulae 6·5–7 (10) × 2·7–3 μ; phialides 6–7 (8) × 2–2·5 μ; conidia globosa aut sub-globosa, glabra, 3–4 × 3–3·5 μ; catenae conidiorum implicatissimae. Habitat in solo, plantario silvatico *Pseudotsugae taxifoliae* (Poir) Britt., Vancouver.

Colonies on Czapek agar plates spreading, thin, at first very pale straw colour, then darkening somewhat to pale fawn with a slight greenish cast, indistinctly zoned, with fimbriate margin; drops not seen; reverse uncoloured. Colonies on Czapek agar slopes at first white and somewhat wet in appearance, then thin matted funiculose, gradually dull greenish fawn, with some white to yellowish overgrowth. Colonies on malt agar growing rapidly, somewhat floccose, white then straw-coloured but with a greenish tone, becoming slightly darker in age and with a fair amount of white aerial mycelium. Penicilli (Pl. 3, fig. 2) short, very compact, usually with four stages of branching, with the elements successively smaller in diameter (as in *Gliocladium*); conidiophores up to 600 μ long, stiff, with granular contents, mostly 5–5·6 μ diam., but occasionally more slender and then bearing small penicilli;; rami 8–12 × 4–5 μ; ramuli 8–10 × 3·5–4 μ; metulae 6·5–7 × 2·7–3 μ, but occasionally up to 10 μ long; phialides crowded, abruptly pointed, 6–7 (8) × 2–2·5 μ; conidia globose to subglobose, smooth-

walled, 3–4 × 3–3·5 μ; conidial chains comparatively short, intricately tangled.

Habitat: forest nursery soil growing Douglas fir, *Pseudotsuga taxifolia*. Description based on a culture sent for identification by Prof. D. C. Buckland, University of British Colombia, Vancouver, who stated that the same species had been isolated several times from forest nursery soil, and occasionally from seed. Culture maintained in the L.S.H.T.M. collection as BB 300.

This species is closely allied to *P. albicans* Bainier, which is, up to the present, the only well-defined species in the group Polyverticillata. The two species are similar in colony colour and in the structure of the penicillus. *P. canadense* differs from *P. albicans* in its shorter phialides, globose instead of almost cylindrical conidia, and long and stiff instead of very short conidiophores.

Cultures of the new species are deposited with the C.B.S., Baarn and the C.M.I., Kew. Dried cultures have been sent to Herb. I.M.I. and Herb. Kew.

REFERENCES

Raper, K. B. & Thom, C. (1949). *Manual of the Penicillia*. London: Baillière, Tindall and Cox.

Thom, C. & Raper, K. B. (1946). *A Manual of the Aspergilli*. London: Baillière, Tindall and Cox.

EXPLANATION OF PLATE 3

Fig. 1. *Aspergillus funiculosus*. Typical heads on smooth stalks. × 800.
Fig. 2. *Penicillium canadense*. Typical penicillus. × 1000.
Fig. 3. *P. atrovenetum*. Irregularly biverticillate penicilli. × 750.
Fig. 4. *P. atrovenetum*. Asymmetric penicillus. × 750.

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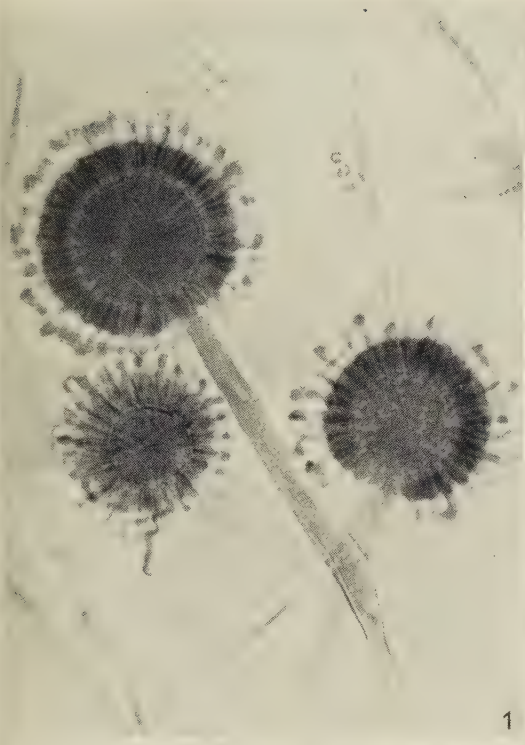

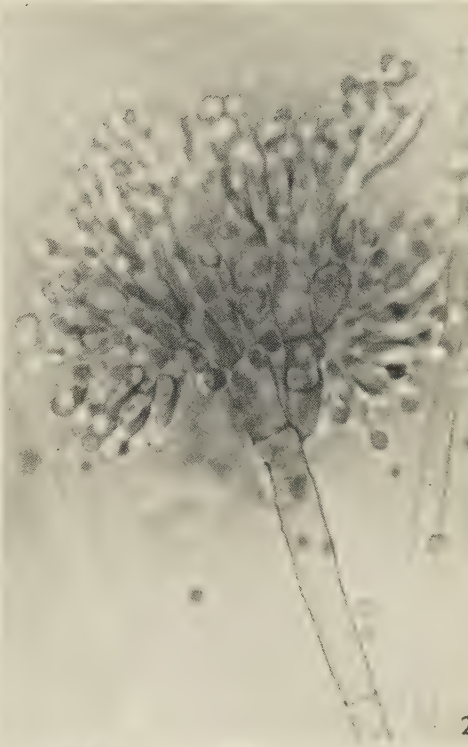

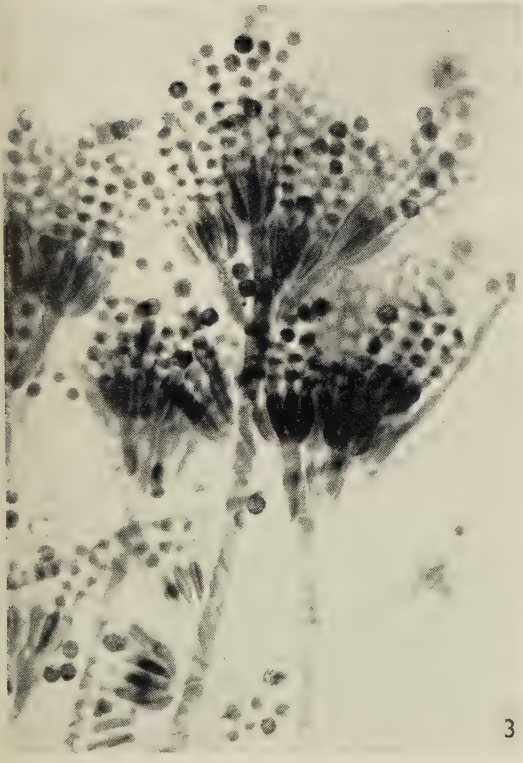

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Trans. Brit. mycol. Soc. **39** (1), 115–124(1956).

A TAXONOMIC INVESTIGATION OF *USTILAGO HYPODYTES* (SCHLECHT.) FR. AND ITS ALLIES

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(With Plate 4 and 1 Text-figure)

The name *Ustilago hypodytes* was rejected as a nomen dubium by Fischer & Hirschhorn (1945). Evidence is here adduced to show that the application of the name is not in doubt. The name is re-instated.

The relationship of *U. hypodytes* with the American species *U. nummularia* Speg., *U. halophila* Speg., *U. spegazzinii* Hirsch. and *U. williamsii* (Griff.) Lavrov is briefly discussed, *U. nummularia* and *U. spegazzinii* being considered synonymous.

Introduction

Stem smut of grasses was first described from the vicinity of Berlin by Schlechtendal (1824). His description, by modern standards inadequate, runs

'*Caeoma hypodytes*. C. acervis distinctis nullis pulvere copioso ex nigro-umbrino culmum ambiente, vaginas replente, sporidiis minutissimis exacte globosis opacis.

Intra vaginas et culmum in *Elymo arenario*.'

The species was later transferred to the genus *Ustilago* by Fries (1832). Since then stem smut has been found throughout Europe and America and generally designated *U. hypodytes*. This name, however, was rejected as a nomen dubium by Fischer & Hirschhorn (1945), who divided the '*U. hypodytes* complex' into the four species *U. spegazzinii* Hirsch., *U. williamsii* (Griff.) Lavrov, *U. halophila* Speg. and *U. nummularia* Speg., all originally described from America. Only *U. spegazzinii* and *U. nummularia* were alleged to occur in Europe.

Search has failed to bring to light any specimens that might have been used for the original description. The claim that *U. hypodytes* is a nomen dubium could therefore be maintained if (*a*) the two alleged European species are shown to be distinct and coexistent in the type locality, and (*b*) the original description is wide enough to cover both species. The first condition is not satisfied by the evidence adduced. First, *U. spegazzinii* allegedly differs morphologically from *U. nummularia* in one character only, the occurrence of polar caps. Considering the small size of the spores it is necessary to ascertain the taxonomic value of the caps. Secondly, the occurrence of *U. nummularia* is based on two specimens, and since Fischer & Hirschhorn examined only some thirty European specimens, an examination of more European material seemed desirable.

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Materials and methods

The material studied included the collections of stem smut in the herbaria of the Royal Botanic Garden, Kew, the British Museum (Natural History), and the Commonwealth Mycological Institute, and of numerous recent British collections. Additional specimens from America, kindly supplied by Prof. G. W. Fischer were also examined. Of some 250 specimens seen, about 170 were British and European, the rest American. (See Appendix for details.)

A routine examination of each collection was made. This consisted of mixing a sample of spores in lactophenol, measuring the diameters of 25 spores at random, and examining individually about 150 spores at random for the presence of polar caps. Colour and nature of the spore surface were also noted.

The fungus was found to grow well on a variety of media, one of the best being beer wort agar (Smith, 1946, p. 173).

Cultures were made from numerous recent British collections on *Elymus arenarius*, *Agropyron repens*, *A. pungens*, and *Bromus erectus*, from American material authenticated by G. W. Fischer as *Ustilago spegazzinii* on *Agropyron trachycaulum* and *Elymus condensatus* and from American material of *Ustilago williamsii* on *Stipa williamsii*, *S. thurberiana* and *Oryzopsis hymenoides*.

Results

The spore

Spore size

No significant differences were found in the European material. The size range is (3) 3·5–5 (6) μ.

Spore colour

No significant differences in colour were noted. Pigmentation does vary slightly, probably with age of development.

Nature of the spore wall

In some collections the surface is obscurely verruculose, but for practical purposes the surface can only be described as smooth.

Polar caps

The caps ('crests' of Fischer & Hirschhorn) have been unobserved or ignored by numerous eminent mycologists (see Fischer & Hirschhorn, 1945, p. 240). Attention was first drawn to them by Fischer & Hirschhorn (1945), and they were independently noted by Ainsworth & Sampson (1950).

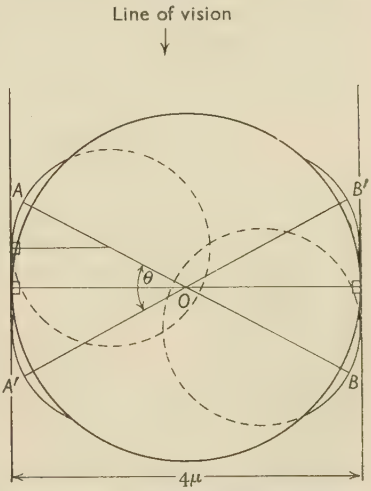
They are of the order of 0·25 μ in height, usually smaller, occasionally larger. They are not obvious under ordinary high power except when unusually large, and are only clearly visible under an oil-immersion lens.

Moreover, in any preparation only a percentage of the spores shows them. The percentage varies between 0 and 65. Two-thirds of the specimens gave percentages between 20 and 50 (Table 1).

Table 1. *Percentages of spores showing caps in different collections of stem smut*

Percentage of spores showing caps	0	1–10	11–20	21–30	31–40	41–50	51–60	60+
No. of specimens	3	11	22	33	32	35	9	1

The caps can only be seen when the poles of the spore are orientated at a suitable angle to the line of vision. Text-fig. 1 is a diagram of a section of a spore through the poles *A* and *B*. At the position indicated by the line *AB* the caps are just out of sight. As the spore revolves through the angle θ the caps are more or less visible and disappear as soon as position *A'B'* is reached.



Text-fig. 1. Diagrammatic section of spore through the polar caps.

The variation then seems to be correlated with the size of the caps. The larger the caps the larger is the percentage of spores showing them at one time.

The reasons for the variability in cap size seems to be at least in part developmental. For example, one specimen on *Elymus* from St Andrews, Scotland, had spores apparently without caps when collected, but material left outside to weather for several weeks developed them. Cap morphology is variable even in a single sample, some spores having smooth caps others crenulated. The crenulations may be due to the breaking of the outer wall of the cap on older spores.

Cultures

The cultures of British material and of *U. spegazzinii* were remarkably similar in morphology, development and rate of growth (Plate 4, figs. 1, 2). No aerial hyphae were produced except a few at the point of inoculation. The cultures were flat smooth and sodden in appearance for about 21 days when abundant sporidia, white in mass, were produced at the surface all over the culture except on a band 4 mm. wide round the growing edge. Colour before sporidia formation was pale Olive Buff to Tilleul Buff (Ridgway, 1912). Rate of growth averaged 4·6 mm. per day at about 60° F.

The cultures of *U. williamsii* were quite different from those described above. A large amount of aerial hyphae wefted into flutings pure white in colour and dry in appearance were produced (Plate 4, figs. 3, 4). Growth rate was much slower, about 2·2 mm. per day at about 60° F.

Conclusions

There is only one European species of stem smut in the morphological sense. The name *U. hypodytes* must therefore be re-instated. It is desirable to choose a lectotype and re-describe the species. Hirschhorn (1947) has already proposed a lectotype in Sydow, Rabenhorst's *Fungi Europ.*, Ser. 2, 3201, and this is a suitable choice. Contrary to her statement this specimen does have the polar caps typical of European material.

Relationships of *U. hypodytes* to *U. spegazzinii*, *U. nummularia*, *U. halophila* and *U. williamsii*:

U. spegazzinii. This must be considered synonymous. The type specimen was not seen, but numerous specimens authenticated by G. W. Fischer have been examined. Cultures from American material differed in no way from cultures from British and European material grown under identical conditions. There is little doubt that they are conspecific.

Table 2. *Stem smut on* Distichlis *from North America*

Specimen	Host	Locality	Range in size of spores (mm.)	Av. size (mm.)	Percentage with caps
Fischer and Law, M.C.B.P.I. 85278	*Distichlis stricta*	Washington	3·4–6	4·2	0
Fischer and Law, M.C.B.P.I. 85291	*D. stricta*	Washington	4–8	5·5	0
Fischer and Law, M.C.B.P.I. 85316	*D. stricta*	Oregon	4–5·5	5	0
Fischer, Sprague and Meiners, M.C.B.P.I. 85415	*D. stricta*	New Mexico	3·5–5·5	4	0
Fischer, Sprague and Meiners, M.C.B.P.I. 85400	*D. stricta*	Utah	4–5·5	4·5	0
Bethel s.n., 1920	*D. spicata*	California	4–5·5	5	0
Bethel s.n., 1916	*D. spicata*	California	4–5·5	4·5	0
Bethel s.n., 1920	*D. spicata*	Nevada	3·5–5·5	4·5	0
Herb. Inst., Speg. 3198	*D. spicata*	S. America	6–8	6·5	0

U. nummularia. The type specimen has been examined and but for the absence of polar caps is identical with *U. hypodytes* and can scarcely be separated on morphological grounds.

U. halophila. The spores of the type specimen (Herb. Inst. Speg. 3198), on *Distichlis* sp. are significantly larger than those of *U. hypodytes* and are without caps. This was the only South American specimen examined, and it remains to be seen whether all the stem smut material on *Distichlis* from South America has spores of a similar order of size. It is perhaps worth noting that eight specimens on *Distichlis* from North America had spores which fell within or overlapped the size-range of those of *Ustilago hypodytes* (Table 2).

For the present it seems best to retain the name *U. halophila* for the smut on *Distichlis* spp., though it may later prove to be only varietally distinct.

Ustilago williamsii. This species is morphologically quite distinct on the host and in culture.

Emended description of *U. hypodytes*:

Ustilago hypodytes (Schlechtendal) Fries, *Systema*, 3, 518, 1832.

Syn: *Caeoma hypodytes* Schlecht., Fl. *Berol.* **2**, 129, 1824.
Ustilago nummularia Speg., *Ann. Mus. Buenos Aires*, **3**, 59, 1902.
U. spegazzinii Hirschhorn, *Notas Mus. La Plata Bot.* **4**, 415–419, 1939.
For additional synonyms see Fischer (1951), p. 149.

Sori in the stems surrounding the internodes, affecting the whole stem except a few basal internodes, at first protected by the leaf-sheath, ultimately naked, occasionally occurring on the axis of a partly formed inflorescence; inflorescence usually completely aborted. Spore mass powdery almost black. *Spores* globose to subovoid, usually with a more or less inconspicuous hyaline smooth or crenulated cap at each pole, Deep Olive Buff to Dark Olive Buff (Ridgway, 1912), smooth to minutely verruculose, (3) 3·5–5 (6) μ diameter.

Lectotype: Rabenhorst's *Fungi Europ.*, Ser. 2, 3201.

Host plants: European—*Agropyron acutum*, *A. cristatum*, *A. repens*, *A. rigidum*, *A. pungens*, *A. junceiforme*, *Bromus erectus*, *Elymus arenarius*, *Lygeum sparteum*. *Festuca gigantea* and *Trisetum flavescens* are doubtful hosts. *Psamma arenaria* is wrongly recorded as a host owing to mis-identifications on some of Sydow's specimens of *Elymus*. American—see Fischer & Hirschhorn (1945).

I am greatly indebted to Dr G. C. Ainsworth for his constant help and advice, and to Prof. J. Caldwell, University of Exeter, in whose laboratories the work was done. Thanks are also due to the curators of the herbaria of the Royal Botanic Garden, Kew, and the British Museum (Natural History) for permission to study the large collections in their care, to Prof. Fischer of Washington, U.S.A., for material he kindly sent and to many other people who kindly loaned or collected specimens.

APPENDIX

List of specimens examined

Abbreviations. The names of herbaria are abbreviated according to the Index Herbariorum, 1952. *A.*, collected by A. Angus and specimen deposited in the herbarium of the University of Exeter. G. B., Great Britain.

European

Agropyron cristatum (L.) Gaertn.

Savulescu and *T. Rayss*, *Herb. Myc. Romanicum*, Fasc. xi, No. 531, Muntenia District, Italy, 15/6/1933 (IMI).

Agropyron junceiforme (A. & D. Löve) A. & D. Löve (*junceum* Auct.)

P. Magnus, Vestergr. Micr. Rar. Sel. 1595, West Kirby, Liverpool, G.B., 3/8/1897 (BM); *E. A. Ellis* s.n., Walton, Suffolk, G.B., 22/7/1945 (Exeter); *coll. unknown*, Kent, G.B., –/7/1911 (E); *A.*, 3 collections, Exmouth, Devon, G.B., 29/7/1950 & 23/9/1950 (Exeter).

Agropyron pungens (Pers.) R. & S.

E. A. Ellis, Wheatacre Marshes, R. Waveney, Norfolk, G.B., 19/7/1944 (Exeter); *F. R. Elliston-Wright*, Yelland, Taw Estuary, Devon, G.B., 23/6/1949 (K); *A.*, Yelland, Devon, G.B., 23/6/1949 (Exeter); *A.*, Dawlish Warren, Devon, G.B., 1/10/1950 (Exeter); *Sydow*, Ustilagineen 307, Istria, Rovigno, –/6/1902 (BM, IMI).

Agropyron repens (L.) Beauv.

H. Diedicke, Sydow Myc. Germ., Thuringen, Erfurt, 26/6/1904 (K); *J. Kunze*, Fungi Sel. Exsicc. No. 15, Katharinienholz, Nr. Islebiam, Eastern Germany, –/1875 (K); *Sydow*, Ustilagineen 106, Lietberfelde, nr. Berlin, –/6/1896 (BM); *Fr. Bubak*, Vestergr. Micr. Rar. Sel. No. 334, Ziegelschanke ad Trebinz, Bohemia, 25/7/1900 (BM); *Leinhart*, Fungi Hungarici No. 104, Budapest, 11/6/1833 (BM); *R. Picbauer*, Petrak Fl. Bohem. et Morav. Exsicc. No. 1988, Lundenburg in Mahren, –/6/1924 (BM); *T. Lind*, Petrak Myc. Gen. 1067, –/7/1916 (IMI); *D. T. Fruley*, Mahren, 1929 (IMI); *A.*, nr. Calais on the Boulogne rd., 1/8/1949 (Exeter); *A.*, St Andrews, G.B., 9/9/1949 (Exeter); *A. Kerr*, Aberdeen, G.B., 8/7/1949 (Exeter); *R. W. G. Dennis*, Edinburgh, G.B., 5/7/1942 (Plant Path. Sta., Edinburgh); *coll. unknown*, Edinburgh, G.B., 19/6/1949 (Plant Path. Sta., Edinburgh); *coll. unknown*, Edinburgh, 27/6/42 (K); *coll. unknown*, Granton, Edinburgh, G.B., –/11/1920 (E); *Johnston*, Herb. Berk., Berwick, G.B., no date (K); *T. E. T. Bond*, Spurn, E. Yorks, G.B., 21/7/49 (Exeter); *W. G. Bramley*, Bolton Percy, Yorks, G.B. 6/7/1936 (BM); *Dr Ellis*, Herb. W. B. Grove, Maghull, Lancs., G.B., 1909 (BIRM); *J. W. Ellis*, Lydiate, Lancs. G.B., 1909 (K); *S. J. Hughes*, Kegworth, G.B., –/8/1943 (IMI); *A.*, 2 collections, Worksop, Notts., G.B., 13/8/1950; *K. Sampson*, Lathkill Dale, Derbyshire, G.B., 1936 (IMI); *A.*, Whitchurch, Shropshire, G.B., 2/9/1950; *M. C. Cooke*, New Cross, –/7/1863 (K); *coll. unknown*, Herb. Berk., King's Cliff, G.B., 31/5/1849 (K); *coll. unknown*, Herb. Berk., Wood Newton, G.B., –/6/1859 (K); *Dillon Weston*, Terrington St-Clement, Norfolk, G.B., 24/6/1949 (Exeter); *E. A. Ellis*, Upper Hellerdon, Norfolk, G.B., 14/6/1949 (IMI); *C. B. Plowright*, Vize Fungi Britanici, King's Lynn, G.B., –/6/1873 (BM); *Dillon Weston*, Haddenham, Isle of Ely, G.B., 21/6/1950 (Exeter); *Dillon Weston*, Cambridge, G.B., 16/6/1950 (Exeter); *Dillon Weston*, Ely, G.B. 16/6/1950 (Exeter); *Dillon Weston*, Earith Bridge, Cambridgeshire, G.B., 6/7/1949 (Exeter); *Dillon Weston*, Eaton Socon, nr. St Neots, G.B., 29/6/1949 (Exeter); *E. A. Ellis*, Brackford, Suffolk, G.B., 23/6/1940 (Exeter); *Dillon Weston*, Harrowden, Beds. G.B., 28/6/1950 (Exeter); *G. C. Ainsworth*, Golf Links, Hanwell, Middlesex, G.B., 29/6/1949 (Exeter); *R. W. G. Dennis*, Great Bookham, Surrey, B.G., 20/7/1946 (K); *A.*, Gloucester, G.B., 11/9/1949; *P. M. Stockdale*, Highbury, Calne, Wiltshire, G.B., 1950 (Exeter); *H. K. Airey-Shaw*, Sandwich Bay, Kent, G.B., 27/6/1949 (Exeter); *A.*, Canterbury, Kent, G.B., 30/7/1949; *A.*, Lyme Regis, Dorset, G.B., 22/7/1950; *A.*, 11 collections, Exeter and environs, G.B., 5 & 6/1949 & 1950; *A.*, Orcombe Pt., Exmouth, G.B. 26/6/1950.

Agropyron rigidum Beauv.

Serebrianikov, Transchel et Sereb. Myc. Rossica, 203, Samarkand, Turkestan, –/7/1910 (K & BM);

Agropyron sp.

P. Dumée, Briosi et Cavara Fung. Paras. Pl. Colt. et Ut. 228, Meaux, France, 1894 (IMI).

Bromus erectus Huds.

Coll. unknown, Herb. H. W. Ravenals, Northants. B.G. –/7/1848 (BM); *G. H. Pethybridge*, nr. Whipsnade, Oxon. G.B., 6/7/1924 (K); *R. Melville*, Middleton, Oxon, G.B., 7/6/1937 (K); *E. T. Roberts*, Streatley, Berks., 15/9/1949 (Exeter); *coll. unknown*, Herb. Broome, King's Cliff, no date (BM); *W. Carruthers*, Evesham, Warwickshire, G.B., no date (BM); *A.*, Cheltenham, G.B., 20/11/1949 (Exeter); *B. Dilnutt*, Cheltenham, G.B.

(Exeter); *K. Sampson*, Cirencester, G.B., 1911 (IMI); *Burkill*, Cirencester, G.B., –/6/1897 (CGE); *Rev. F. Dennis*, Epsom Downs, G.B. 27/5/1945 (IMI); *R. W. G. Dennis*, Epsom Downs, G.B., 3/7/1949 (Exeter); *coll. unknown*, Herb. Berk., Chalk Downs, Kent, G.B., 30/6/1876 (K); *H. K. Airey-Shaw*, Kew, 14/6/1949 (Exeter).

Elymus arenarius L.

Bornmüller, Mus. Hist. Nat. Vindoben 2072, Germany, no date (IMI); *G. Lagerheim*, Vestergr. Micr. Rar. Sel., Scania, Suecia, –/8/1896 (BM); *Johanson*, Eriksson Fung. Parasit. Scand. 251, Halmstad, Suecia, 30/7/1830 (BM); *Sydow*, Berlin, –/8/1884 (BM); *Sydow*, Myc. Germ. 1164, Pommern bei Zingst, 14/8/1912 (K); *Sydow*, Myc. Germ. Westend bei Berlin –/7/1884 (K); *Sydow*, Rabenhorst Winter Fung. Eur., nr. Berlin –/10/1884 (K); *A. G. Eliasson*, Petrak Myc. Gen. 1069, Malen bei Bastad, Schweden, 7/8/1918 (IMI); *A. G. Eliasson*, Petrak Myc. Gen. 398, Malen bei Bastad, Schweden, –/7/1927 (IMI); *A. Lugdwig*, Fl. of Kurland, Libau, 10/8/1927 (IMI); *G. Samuelsson*, Mus. Hist. Nat. Vindoben 2072 Suecia, no date (K & IMI); *G. H. Pethybridge*, Aberdeen, G.B., 8/7/1924 (K); *A. Kerr*, 2 colls., Aberdeen, G.B. 6/3/1949, and 8/7/1949 (Exeter); *H. Macfarlane*, 2 colls., Aberdeen, G.B., 10/7/1938 and –/9/1940 (IMI); *J. Sadler*, Cooke Fungi Brit. Exsicc. (2) 433, Broughty Ferry, G.B., no date (K & IMI); *McIntosh*, St Andrews, G.B., 25/6/1949 (Exeter); *Wilkinson*, St Andrews, G.B., 11/5/1889 (BIRM); *coll. unknown*, St Andrews, G.B., 1919 (E); *A.*, 2 colls., St Andrews, G.B., 19/8/1948 and 9/9/1949; *C. E. Foister*, Montrose, G.B., –/8/1943 (Pl. Path. St Edinburgh); *T. E. T. Bond*, Spurn, E. Yorks. G.B., 21/7/1949 (Exeter); *S. P. Rowlands*, Sandhills, St Anns, Lancs., G.B., 2/7/1946 (K); *M. R. Brown*, Hunstanton, Norfolk, G.B., 29/7/1938 (CGE); *J. Webster*, Hunstanton, Norfolk, G.B., 16/8/1949 (Exeter); *P. E. Rumbelow*, Herb., Ellis, Gt Yarmouth, G.B., –/8/1946 (Exeter); *coll. unknown*, comm. L. E. Hawker, Berrow, Somerset, G.B. 8/7/1946 (K).

Elymus europaeus L.

Roumeguère Fungi Gall. Exsicc., Brussels, Belgium, no date (K).

Lygeum spartum Loefl. ex L.

Loscos, Rabenhorst Fung. Eur., Castelseras, Spain, no date (K & BM); *V. Beltrani*, Thümen Myc. Universalis 930, Sicily 1877, (K); *Marcucci*, Unio. Itin. Crypt. 1866, no date (K).

Stipa pennata L.

Fruley, Petrak Myc. Gen. 1080, nr. Auspitz, Mahren, –/4/1930 (IMI).

American

Agropyron caninum (L.) Beauv.

Fischer, M.C.B.P.I. 85252, Pullman, Washington, 7/7/1944 (IMI).

Agropyron cristatum (L.) Gaertn.

Fischer, M.C.B.P.I. 85259, Pullman, Washington, 7/7/1944 (IMI); *Fischer, Law and Menzies*, M.C.B.P.I. 85177, Goldendale, Washington, 23/6/1943 (IMI).

Agropyron dasystachyum (Hook.) Scribn.

Fischer, M.C.B.P.I. 85227, Pullman, Washington, 7/7/1944 (IMI).

Agropyron inerme (Scribn. & Smith) Rydb.

Fischer, M.C.B.P.I. 85222, Pullman, Washington, 7/7/1944 (IMI); *Fischer & Law*, M.C.B.P.I. 85279, Washington, 19/6/1945 (IMI).

Agropyron intermedium (Host) Beauv.

Fischer, M.C.B.P.I. 85225, Pullman, Washington, 7/7/1944 (IMI).

Agropyron occidentale Scribn.

Griffiths and Lange, West American Fungi, Billings, Montana, –/7/1900 (BM).

Agropyron repens (L.) Beauv.

Russel, Univ. Toronto Crypt. Herb. 638, Saskatoon, Sask., 16/6/1938 (IMI); *Hafenrichter*, M.C.B.P.I. 85232, Craigmont, Idaho, –/6/1944 (IMI); *Fischer and Law*, M.C.B.P.I. 85314, Grangeville, Idaho, 28/6/1945 (IMI); *Fischer and Law* M.C.B.P.I. 85305, Walla Walla, Washington, 30/6/1945 (IMI); *Fischer*, M.C.B.P.I. 85213, Union, Oregon, 10/8/1944 (IMI); *Fischer and Holton*, M.C.B.P.I. 85343, Twin Falls, Idaho, 22/7/1945 (IMI).

Agropyron sibiricum (Willd.) Beauv.

Fischer M.C.B.P.I. 85223, Pullman, Washington, 10/7/1944 (IMI).

Agropyron trachycaulum (Link). Malte

Kreizinger, M.C.B.P.I. 85437, Pullman, Washington, 10/7/1944; *Fischer*, M.C.B.P.I. 85224, Pullman, Washington, 10/7/1944 (IMI); *Fischer*, M.C.B.P.I. 85257, Pullman, Washington, 7/7/1944 (IMI).

Distichlis spicata (L.) Greene

Coll. Mic. Univ. Nac. de la Plata Mus. Inst. Speg. 3198 (type of *U. halophila* Speg.), Santa Fe, Rufino, –/12/1900; *Bethel*, U.S. Dep. Agr. B.P.I., Long Beach, California, –/6/1916 (K); *Griffiths and Morris*, West Amer. Fungi 235, Quinn River Crossing, Nevada, –/7/1901 (BM); *Bethel*, U.S. Dep. Agr. B.P.I., Santa Barbara, Calif. 4/4/1920 (K); *Bethel*, U.S. Dep. Agr. B.P.I., Minden, Nevada, 26/5/1920 (K); *Griffiths*, Vestergr. Micr. Rar. Sel. 898, Roswell, New Mexico, 9/5/1903 (BM).

Distichlis stricta (Torr.) Rydb.

Fischer & Law, M.C.B.P.I. 85316, Baker, Oregon, 29/6/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85291, Corfu, Washington, 18/6/1945 (IMI); *Fischer & Law* M.C.B.P.I. 85278, Dry Falls State Park, Washington, 19/8/1945 (IMI); *Fischer, Sprague & Meiners*, M.C.B.P.I. 85400, Manti, Utah, 8/6/1947; *Fischer, Sprague & Meiners*, M.C.B.P.I. 85415, San Antonio, New Mexico, 12/6/1947.

Elymus canadensis L.

Fischer, M.C.B.P.I. 85253, Pullman, Washington, 7/7/1944 (IMI).

Elymus condensatus Presl.

Fischer, Sprague & Meiners, M.C.B.P.I. 85436, Ryepatch, Nevada, 23/6/1947; *Griffiths & Morris*, West Amer. Fungi, Quinn River Crossing, Nevada, 20/7/1901.

Elymus dahuricus Turcz.

Fischer, M.C.B.P.I. 85255, Pullman, Washington, 7/7/1944 (IMI).

Elymus glaucus Buckl.

Fischer, M.C.B.P.I. 85220 & 85226, Pullman, Washington, 7/7/1944 (IMI); *Fischer & Law*, M.C.B.P.I. 85310, Dayton, Washington, 20/6/1945 (IMI)

Elymus striatus Willd.

Griffiths & Large, West Amer. Fungi, Billings, Montana, –/8/1900 (BM).

Elymus triticoides Buckl.

Fischer & Law, M.C.B.P.I. 85308, La Grande, Oregon, 29/6/1945 (IMI).

Oryzopsis hymenoides (Roem. & Schult.) Ricker

Fischer, Sprague & Meiners, M.C.B.P.I. 85398, Glendale, Utah, 9/6/1947; *Stevenson* 711, Beaver Creek, Sask., 28/8/1938 (IMI); *Fischer & Holton*, M.C.B.P.I. 85344, Glens Ferry, Idaho, 22/7/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85277, Burke, Washington, 19/6/1945 (IMI); *Fischer, Sprague & Meiners*, M.C.B.P.I. 85438, Winnemucca, Nevada, 23/6/1947.

Stipa comata Trin. & Rupr.

Russel, Saskatoon, 18/7/1938 (IMI); *T. A. Williams*, Bigstone, S. Dakota, 15/6/1895 (U.S. Nat. Mus. & K.); *Fischer, Sprague & Meiners*, M.C.B.P.I. 85434, West of Wabuska, Nevada, 23/6/1947; *Fischer & Law*, M.C.B.P.I. 85296 & 85275, La Crosse, Washington, 18/6/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85276, Burke, Washington, 19/6/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85322, Baker, Oregon, 29/6/1945 (IMI); *Fischer*, M.C.B.P.I. 85221, Asotin, Washington, 12/6/1944 (IMI); *Brenckle*, Fungi Dakotensis 675, Northville, S. Dakota, 18/7/1929 (IMI); *Fischer & Law*, M.C.B.P.I. 85208, Pasco, Washington, 24/6/1943 (IMI); *Fischer*, Wilsall, Montana, M.C.B.P.I. 85349, 31/7/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85335, Baker, Oregon, 29/6/1945 (IMI).

Stipa eminens Cav.

Holway, U.S. Dep. Agr. B.P.I., Berkley, California, 18/5/1894 (K).

Stipa pulchra Hitchc.

Timmon, M.C.B.P.I. 85237, San Fernando, California, 16/5/1944 (IMI); *L. Bonar & V. P. Mentzer*, Berkley Hills, California, 29/6/1937 (K).

Stipa setigera J. Presl.

Bartholomew, Fungi Columbiani 4794, Coronado Is, Mexico, 8/7/1915 (IMI).

Stipa spartea Trin.

Griffiths, U.S. Dep. Agr. B.P.I., Scatterwood, S. Dakota, –/7/1896 (IMI & BM); *Stewart*, Clinton Econ. Fungi, Ames, Iowa, 9/7/1892 (K & BM); *Waite*, Clinton Econ. Fungi, Urbana, Illinois, 21/6/1888 (K & BM).

Stipa thurberiana Piper

Fischer, M.C.B.P.I. 85249, Burns, Oregon, 9/6/1944 (IMI); *Fischer*, M.C.B.P.I. 85219, Haines, Oregon, 10/6/1944 (IMI); *Fischer & Law*, M.C.B.P.I. 85289, Othello, Washington, 18/8/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85321, Baker, Oregon, 29/6/1945 (IMI); *Fischer & Law*, M.C.B.P.I. 85285, Coulee City, Washington, 19/6/1945 (IMI).

Stipa viridula Trin.

Shirley, Brenckle Fungi Dakotensis 524, Manfred, N. Dakota, 20/6/1922 (IMI); *Griffiths*, U.S. Dep. Agr. B.P.I., Brookings, S. Dakota, no date (K); *Bethel*, U.S. Dep. Agr. B.P.I., Pagosa Springs Colorado, 24/8/1921 (K).

Stipa williamsii Scribn.

Fischer & Law, M.C.B.P.I. 85271, Washington Boundary, 28/6/1945 (IMI); *coll. unknown*, M.C.B.P.I. 85269, Northport, Washington, 20/6/1845 (IMI).

Stipa sp.

Bethel, U.S. Dep. Agr. B.P.I., Al Tahoe, Californio, 3/9/1921, (K); *Bethel*, U.S. Dep. Agr. B.P.I., Truckee, California, 22/8/1920 (K); *Bethel*, U.S. Dep. Agr. B.P.I., Santa Fe, New Mexico, 10/10/1920 (K); Col. Mic. Univ. Nac. de La Plata Mus. Inst. Speg.

3619 (type of U. nummularia Speg.) –/2/1900 (fragment in K); *Paradi* 3689, Col. Mic. Univ. Nac. de La Plata Mus. Inst. Speg. 4596, Buenos Aires, no data; *Ruiz Leal* 11034 Col. Mic. Univ. Nac. de La Plata Mus. Inst. Speg., Mendoza, 21/12/1947.

Africa and Australia

Diplachne fusca (L.) Beauv.

Schweinfurth, Nile Delta, Egypt, –/5/1880 (K).
Lygeum spartum Loefl. ex L.
L. Trabut, Roumeguère, Fungi Gall. Exsicc., Oran, Algeria, –/4/1886 (K); *René Maire*, Vestergr. Micr. Rar. Sel. 1205, Algeria, 18/4/1906 (BM).

Stipa aristiglumis F. Muell.

Coll. unknown comm. Langdon via Dep. Agr. Queensland, Australia, 1949 (IMI).

Stipa falcata D. K. Hughes

Burbridge, Marrumbidgee River, Australian Capital Territory, 13/1/1948 (K).

REFERENCES

Ainsworth, G. C. & Bisby, G. R. (1945). *A dictionary of the fungi.* Kew, Surrey.
Ainsworth, G. C. & Sampson, K. (1950). *The British smut fungi.* Kew, Surrey.
Fischer, G. W. (1951). *The smut fungi.* New York.
Fischer, G. W. & Hirschhorn, E. (1945). A critical study of some species of *Ustilago* causing stem smut on various grasses, *Mycologia*, **37**, 236–266.
Fries, E. (1832). *Systema Mycologicum*, **3**, 518.
Hirschhorn, E. (1947). Critical observations on the Ustilaginaceae. *Farlowia*, **3** (1), 73.
Ridgway, R. (1912). *Color standards and color nomenclature.*
Schlechtendal, D. F. L. (1824). *Flora Berol.* **2**, 129.
Smith, G. (1946). *An introduction to industrial mycology.* London.

EXPLANATION OF PLATE

Fig. 1. *Ustilago williamsii*: 22-day-old culture on beer wort agar; from Fisher & Law specimens M.C.B.P.I. 85269 on *Stipa williamsii*. × 1·5.
Fig. 2. *U. williamsii*: 46-day-old culture on beer wort agar; from Fischer & Law specimens M.C.B.P.I. 85269 (centre) and 85271 (right) on *Stipa williamsii* and M.C.P.B.I. 85335 (left) on *S. thurberiana*.
Fig. 3. *U. hypodytes*: 34-day-old culture on beer wort agar; from Angus s.n. on *Agropyron repens*. × 1·6.
Fig. 4. *U. hypodytes*: 46-day-old-cultures on beer wort agar; from left to right Kerr s.n., Kerr s.n. bis, and Angus s.n., on *Elymus arenarius*.

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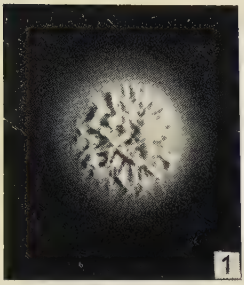

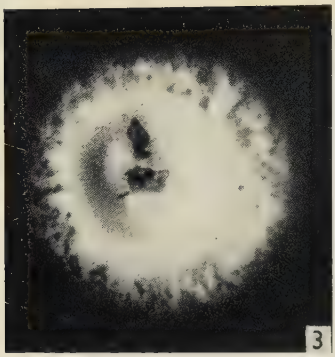

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STUDIES ON SOIL CHYTRIDS. I. *RHIZIDIUM RICHMONDENSE* SP.NOV. AND ITS PARASITES

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(With 9 Text-figures)

The saprophytic soil chytrid *Rhizidium richmondense* sp.nov., which grows on cellulosic baits, is described together with its parasites *Chytridium suburceolatum* sp.nov., *C. parasiticum* sp.nov., and *Septosperma rhizophidii* Whiffen. *Rhizidium richmondense* is considered essentially inoperculate, but a portion of the sporangium wall may occasionally separate at dehiscence. *C. parasiticum* also parasitizes *Rhizophlyctis* sp. and *Entophlyctis* sp., and *Septosperma rhizophidii* parasitizes *Rhizophlyctis* sp. Further, *Chytridium parasiticum* hyperparasitizes *C. suburceolatum* and *Septosperma rhizophidii*.

Although numerous soil chytrids have been reported from the New World, little is known of their occurrence in Great Britain. Karling's (1946) *Rhizophydium keratinophilum* from Irish soil is one of the few recorded in recent years.

Materials and methods

Soil taken from the humus layer in a plantation of sweet chestnut in Richmond Park, Surrey, England, has been used exclusively in the present investigation.

In the laboratory about 3 g. of soil is placed in each of a number of Petri dishes and just covered with charcoal water or soil extract solution. Suitable baits for the growth of saprophytic chytrids are then introduced and the dishes left at room temperature (approximately 20° C.).

Charcoal water (Couch, 1939) is prepared by boiling 2 g. of powdered animal charcoal with 500 ml. of glass-distilled water for 1 hr. The solution is filtered and autoclaved. Soil extract solution (Hanson, 1945) is prepared by placing 280 g. of soil in contact with 1 l. of distilled water for 2 days, followed by filtration and autoclaving.

The baits used were Cellophane, grass leaves, and the epidermis stripped from the succulent bulb scales of onions. Methods of preparation of the baits follow closely those of previous investigators. The Cellophane (Haskins, 1939) was P.T. 300 as supplied by British Cellophane Ltd. and was autoclaved in distilled water before use. The grass used (Berdan in Couch, 1939) was *Poa annua*, young leaves being selected, and the two halves of each folded leaf separated. The leaves were bleached in absolute alcohol, which was then removed by transferring to boiling water. They were then autoclaved before use. So far as the onion strippings are concerned, the epidermis from the convex surface of the innermost fleshy scales has proved most suitable. The strippings are boiled in water to remove

most soluble substances, and are then autoclaved in water. During manipulation they normally float with the inner (broken) surface downwards. These floating strippings prove a particularly suitable bait to use, as chytrids develop on the undersurface and are relatively free from bacterial and other contaminants.

Within 2 days chytrids appear on the baits, which are removed for microscopic examination as required. By mounting in fresh charcoal water, sporangial dehiscence can often be induced.

Results

Rhizidium richmondense sp.nov.

This species grows in great quantity on Cellophane and onion epidermis baits and was regularly obtained from every soil collection made throughout the year 1954.

The encysted zoospore first develops a single rhizoidal axis, the branches from which spread over the substratum (Fig. 1 a). The body of the young thallus has become somewhat elongated by the end of 24 hr. (Fig. 1 *b*, *c*); and about 12 hr. later an apiculus, developed from an unexpanded part of the zoospore cyst, is apparent, usually at a point diametrically opposite the insertion of the rhizoidal system (Figs. 1 *d*, *e*). Further increase in size of the sporangium occurs and refractive particles appear in the cytoplasm.

At maturity the sporangium is globose or subspherical, the height being approximately the same as the width. These dimensions vary from 9·5 to 35 μ. Occasionally the sporangium instead of being globose appears ovoid when viewed from above, the longer axis in this aspect measuring up to 41 μ. As in many chytrids a zone of clear cytoplasm occurs, in addition to the mass of zoospores within the mature sporangium. In this chytrid the clear cytoplasm is found below the apiculus and between it and the mass of zoospores. Further, this zone may extend considerably on either side below the apiculus (Fig. 1 *h–j*). Fay (1947) has recorded a similar zone in the mature sporangium of *Chytriomyces spinosus*, in which species it may be even more pronounced and occupy the whole top half of the sporangium.

The rhizoidal system of *Rhizidium richmondense* is very extensive, a lateral spread of 235 μ being figured (Fig. 1 *p*); and a knob can sometimes be seen projecting into the sporangium at the rhizoidal insertion (Fig. 3 *h*, *n*). This structure becomes apparent in all sporangia after dehiscence.

The thalli reach maturity in approximately 7 days at 20° C., and mounting the substratum in fresh charcoal water usually results in spore discharge, up to 90% of the mature sporangia present undergoing dehiscence. Hundreds of dehiscing sporangia have been observed on different occasions, and the behaviour at the moment of dehiscence has been observed nineteen times. What precisely happens at this moment was only seen where a clear optical section could be focused through the apiculus. A bulging of the sporangium wall around the base of the apiculus was a characteristic preliminary (Fig. 1 *k*, *l*). This phase, easily recognized with a little practice, lasts for a few seconds. My interpretation is that the sporangium wall around the base of the apiculus is being stretched and thinned during this phase, and the rate and manner in which this proceeds deter-

mines subsequent behaviour. Although a symmetrical bulging is figured (Fig. 1 *l*) and is most typical, it may develop in such a manner as to give the apiculus a pronounced tilt to one side. A sudden jerk then occurs at one side of the apiculus in the region of bulging, and the zone of clear cytoplasm, closely followed by the zoospores, is seen passing out rapidly through a wide circular orifice. In my experience of dehiscence in typical inoperculate chytrids, as exemplified by members of the genera *Rhizophidium* and *Rhizophlyctis*, there is no sudden jerk, but rather a gradual bulging outwards of the limiting membrane of the papilla apex, no doubt associated with its liquefaction, as the contents of the sporangium begin to ooze out. I interpret the jerk observed as due to a sudden tearing of the sporangium wall in a wide arc across the apex, but to one side of the apiculus. Examination of empty sporangia shows that the apiculus can be recognized near the rim of the exit orifice; and this is the typical condition (Fig. 3 *a–d*).

By the time the zoospores have formed a spherical mass at the sporangium mouth (Fig. 2 *d*), the clear cytoplasm can no longer be seen. The zoospores then appear to be carried out passively some distance from the sporangium so that in optical section they appear as a crescent-shaped mass, part of an invisible sphere with a diameter considerably larger than that of the zoospore mass when it was first liberated. Eventually the zoospores begin to vibrate and finally to move actively and violent swarming occurs in a definite spherical volume. During this stage the zoospores can re-enter the sporangium. Collective swarming proceeds for up to 20 min. and during this time the zoospores can escape from any point on the surface of the swarm-vesicle, the outline of which is never actually visible but can be clearly deduced from the movements of the zoospores. Using a fine pipette the swarming mass can be distorted by suction, but it usually remains attached to the sporangium orifice and springs back into normal position and shape when the suction is released. However, suction may completely detach the vesicle from the sporangium so that it floats away with its milling zoospores. Rough handling of the swarming mass in this way can result in bursting of the vesicle, and the zoospores then escape simultaneously in all directions. If soil particles or other detritus obstruct the normal development of the swarming vesicle, it may become very irregular and even break up into several smaller vesicles in each of which swarming occurs. This is evidence against the existence of a rigid membrane surrounding the zoospores. In all these cases motile and apparently normal zoospores are released.

Zoospores of several different types have been obtained from different sporangia of *Rhizidium richmondense*. A variation in the size of the oil globule is the main difference between them; and in addition to the oil globule refractive regions may occur in the cytoplasm (Fig. 1 *m*, *o*). The zoospores are oval in motion with a lateral oil globule, and 3–3·3 μ long by 2·5–3 μ wide (Fig. 1 *m*), or spherical and 3–3·3 μ in diameter (Fig. 1 *n*, *o*). The posterior flagellum is 18–23 μ long. Despite these minor differences in zoospore character, the consistent occurrence of the bulging of the sporangium wall around the base of the apiculus prior to their liberation is evidence for a single species being involved, although this work has not been carried out with unifungal cultures.

Some variants of the dehiscence mechanism will now be considered. In the example shown in Fig. 3*h–m*, the sporangium burst in such a way that an apical portion of the sporangium wall including the apiculus was torn free. In the sporangium illustrated in Fig. 3*e*, symmetrical bulging took place, and two almost simultaneous jerks occurred, one on each side of the apiculus, which was apparently shorn off by two lateral tears and pushed to one side by the emerging sporangial contents. The apiculus was seen to be a complete and separate structure floating free from the rim of the sporangium after dehiscence. In the sporangia shown in Fig. 3*f*, *g*, although observations were not made at the precise moment of dehiscence, it appears that the zoospores emerged through a region of the wall remote from the apiculus, in spite of the fact that clear cytoplasm was seen in the normal position before dehiscence in the sporangium shown in Fig. 3*g*.

It thus appears that zoospores are released by the occurrence of a single or multiple tear near the apiculus; which usually leaves the sporangium intact, but can result in the removal of the apiculus or the apiculus and part of the sporangium wall. In other exceptional cases tearing of a part of the sporangium wall remote from the apiculus can allow zoospore emergence.

In spite of the fact that occasionally a portion of the sporangial wall may separate as a lid-like structure, I regard this chytrid as essentially inoperculate. I base this view on the bulging of the sporangium prior to dehiscence, which never appears to be a feature of operculate chytrids.

The monocentric, eucarpic and interbiotic form places this chytrid in the Rhizidiaceae of Sparrow's classification; and as the sporangium develops from the body of the zoospore, and the rhizoids arise from one point, the genus *Rhizidium* is indicated. It resembles *R. ramosum* Sparrow to some extent in outward appearance, but in that species there is a terminal discharge papilla. In regard to the relation between the apiculus and the mode of dehiscence, a parallel occurs with *Blyttiomyces spinulosus* Bartsch (1939), in which, however, the zoospores are liberated singly from the sporangium. The traditional conception of *Rhizidium* is that in this genus the sporangium wall develops by even enlargement of the zoospore cyst. However, in *R. richmondense*, a portion of the cyst fails to enlarge and forms the apiculus. Karling (1949) has also reported two species of *Rhizidium* in which uneven enlargement of the zoospore cyst may occur. I suggest that our concept of *Rhizidium* be widened even further to include dehiscence by splitting of the sporangium wall as well as dehiscence by papillae.

Rhizidium richmondense sp.nov. (Figs. 1–3)

Thallus monocentricus, eucarpicus, interbioticus, consistens ex globoso aut subgloboso sporangio et ramosis rhizoidibus, uno puncto annexis. Sporangium 9·5–35 μ diam., cum terminali obtuso apiculo, dehiscens rupto sporangii pariete. Zoosporae globosae, 3–3·3 μ diam., aut ovatae 3–3·3 × 2·5–3 μ, cum una olei guttula et flagello posteriore, 18–23 μ. Zoosporae, qua dehiscit sporangium, ibi examen faciunt, in unum conglomeratae, mox singulatim effugientes. Restantes sporae non observatae sunt. *Hab.* ex humo in Richmond Park, Surrey, Anglia, in epidermide *Allii cepae* et 'Cellophane'. Typus: Herb. I.M.I. 60445.

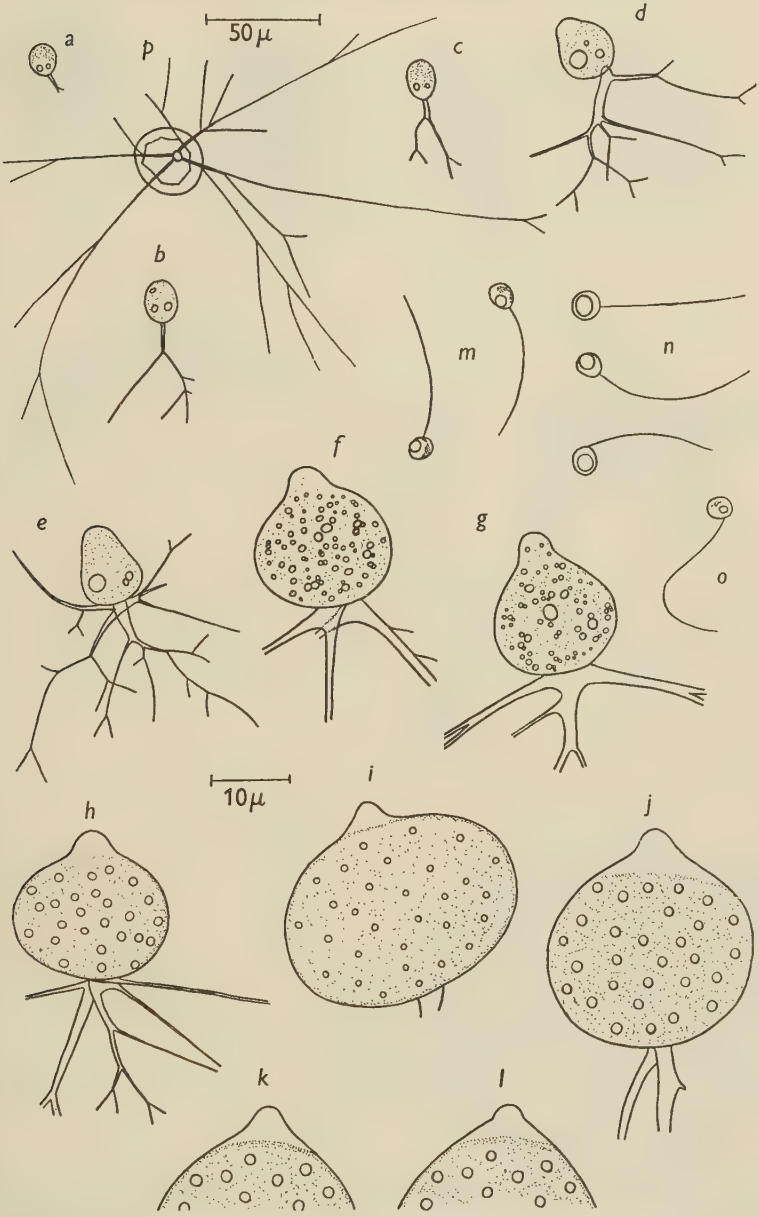

Fig. 1. *Rhizidium richmondense* sp.nov. *a*, young sporangium with rhizoidal axis developed; *b*, *c*, young sporangia after 24 hr. development; *d*, *e*, young sporangia after 36 hr. development; *f*, *g*, sporangia approaching maturity; *h–j*, mature sporangia; *k*, *l*, same mature sporangium before and after bulging round the base of the apiculus immediately prior to dehiscence; *m–o*, zoospores; *p*, dehisced sporangium from above. (All drawings at same scale except *p*.)

Fig. 2. *Rhizidium richmondense* sp.nov. Collective swarming sequence, based on measurements, camera lucida drawings, and free-hand sketches. *a*, mature sporangium; *b*, *c*, zoospore mass emerging with clear cytoplasm visible above it; *d*, spherical zoospore mass at the sporangium mouth; *e*, *f*, zoospores being carried out passively; *g*, collective swarming and escape of zoospores; *h*, later stage in collective swarming with deduced volume of swarm-vesicle indicated by shading.

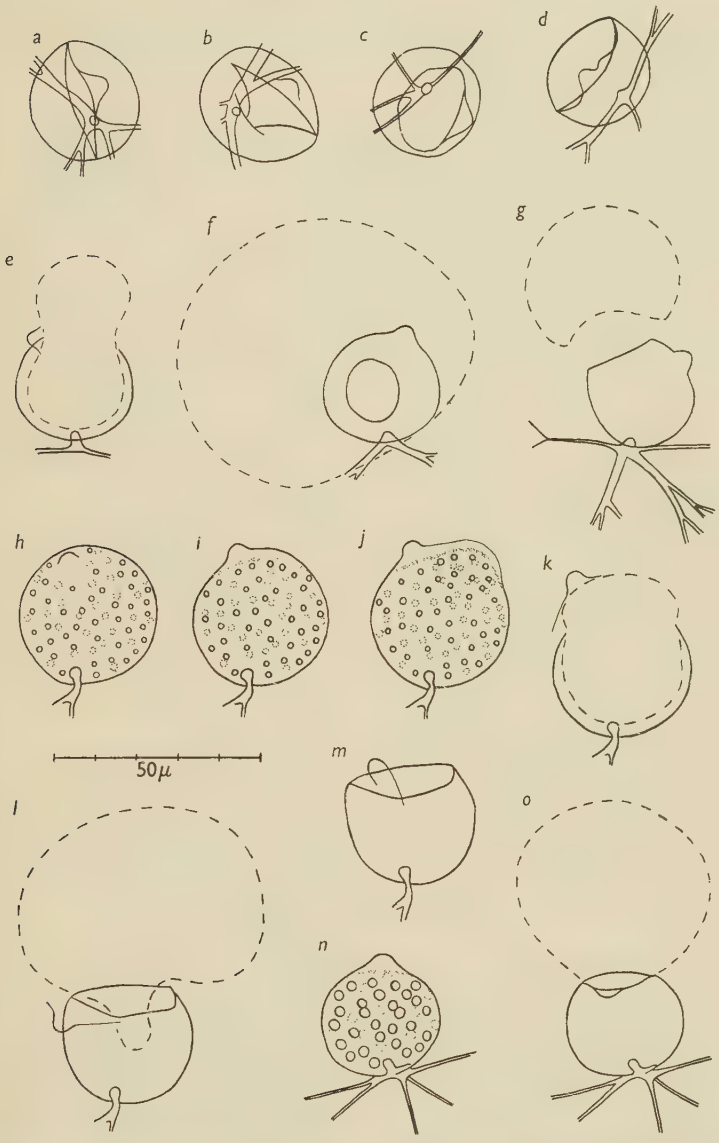

Fig. 3. *Rhizidium richmondense* sp.nov. Sporangium dehiscence. Where relevant, outline of zoospore mass or deduced volume of swarm-vesicle is indicated by an interrupted line. *a–d*, dehisced sporangia; *e*, dehiscing sporangium showing shorn-off apiculus to left of exit orifice; *f*, *g*, zoospore emergence has occurred through a region of the sporangium wall remote from the apiculus; *h–m*, a series of drawings of a sporangium showing dehiscence resulting in an apical portion of the sporangium wall being torn free; *n*, *o*, same sporangium before and after zoospore emergence. In *o*, the apiculus is visible on the rim of the exit orifice.

Chytridium suburceolatum sp.nov.

This chytrid occurs as a parasite of *Rhizidium richmondense*, growing on onion epidermal strippings.

The thallus is monocentric and consists of an extramatrical suburceolate sporangium and an intramatrical penetration tube from which rhizoids develop to a varying extent. Development is very rapid and zoospores are often released after 3 days from the time of plating out the soil.

Early developmental stages illustrated (Fig. 4*a–c*) show the oil globule of the zoospore is retained until the main axis of the rhizoidal system is formed. Later the sporangial contents have a fine granular appearance (Fig. 4*j*, *l*), but lack the refractive particles characteristic of most chytrids prior to the appearance of the zoospore oil globules. The wall of the mature sporangium is thin when rapid development has taken place; but it may be up to 0·9μ thick and develop a light brown coloration in sporangia which take longer to come to maturity. The mature sporangium is suburceolate with a long axis 4–16μ, and contains oil drops 0·6–0·8μ in diameter (Figs. 4*f–h*). Normally there is a single terminal operculum, but very occasionally sporangia with two opercula have been observed (Fig. 4*m*).

On dehiscence an initial vigorous emergence of the zoospores occurs through the orifice left after the separation of the lid-like operculum; and subsequently zoospores escape singly, after a period of motility inside the sporangium. Zoospores are spherical, 2·0–2·5μ in diameter, with a posterior flagellum 14μ, and a lateral oil drop 0·6–0·8μ. Other minute refractive dots occur in the zoospore cytoplasm. Zoospore motion is of the type often referred to as 'hopping and dancing'.

It has proved extremely difficult to demonstrate a rhizoidal system of the chytrid host, in order to ascertain its identity. This is partly due to the fact that the host is attacked while it is still immature, but a possibility remains that parasitic attack prevents normal development of the host rhizoidal system. In favourable cases, however, it has been possible to be certain that the host is *Rhizidium richmondense*.

Chytridium suburceolatum sp.nov. (Fig. 4)

Thallus monocentricus, eucarpicus, epibioticus, consistens ex late suburceolato sporangio, 4–16μ longo, cum uno operculo apicali, infrequenter duobus. Systema ex rhizoidibus intramatricale consistens ex principali axe a quo oriuntur tenuia rhizoidea. Zoosporae globosae, 2–2·5μ diam., cum una olei guttula et flagello posteriore 14μ. Restantes sporae non observatae sunt. Hab. in vivente *Rhizidio richmondensi* Willoughby ex humo in Richmond Park, Surrey, Anglia. Typus: Herb. I.M.I. 60443.

For a discussion of the taxonomic position of this chytrid see p. 135. The hyperparasitic attack of *Chytridium suburceolatum* by *C. parasiticum* is also described below.

Chytridium parasiticum sp.nov.

This species occurs as a parasite of *Rhizidium richmondense*, *Rhizophlyctis* sp. and *Entophlyctis* sp.; and hyperparasitic on *Chytridium suburceolatum* and *Septosperma rhizophidii* Whiffen.

A complete study of the species of *Rhizophlyctis* and *Entophlyctis* parasitized has not been made. Consequently, they will be referred to as *Rhizophlyctis* sp. and *Entophlyctis* sp. Both have a number of rhizoidal axes inserted on

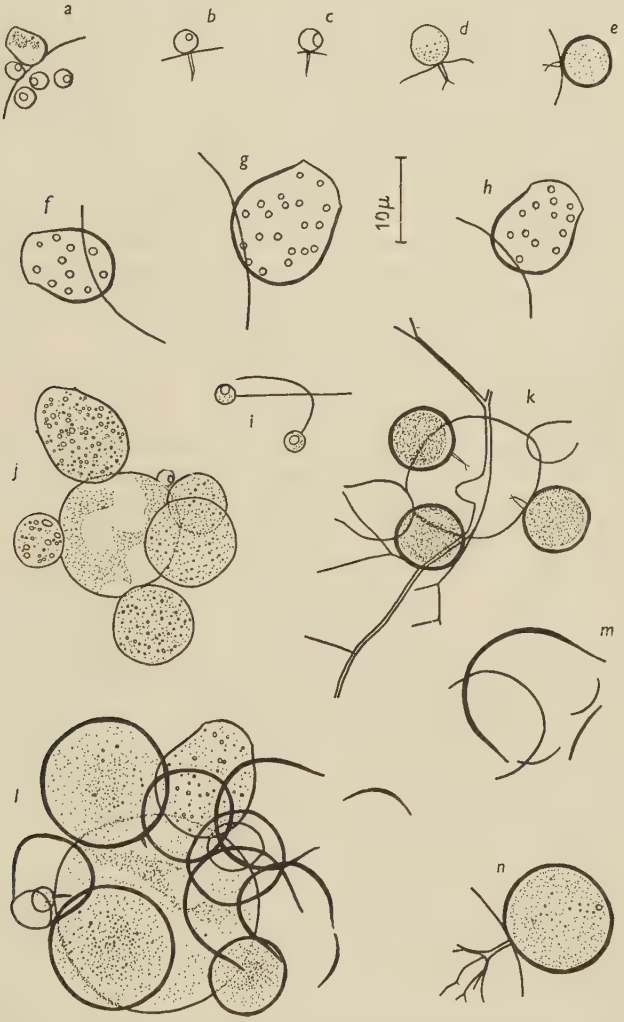

Fig. 4. *Chytridium suburceolatum* sp.nov. parasitic on *Rhizidium richmondense*. *a*, encysted zoospores; *b*, *c*, young thalli with oil globule of the zoospore still visible; *d*, *e*, young thalli. *f–h*, mature sporangia; *i*, zoospores; *j*, young sporangia on a sporangium of the host; *k*, sporangia on a host sporangium, the rhizoidal system of which is visible (stained with cotton-blue). *l*, sporangia in various stages of development on a host sporangium (free opercula are seen right); *m*, dehisced sporangium showing two opercula; *n*, sporangium showing penetration tube and rhizoidal system, stained with cotton-blue.

the sporangium. *Entophlyctis* sp. has a long exit tube, while *Rhizophlyctis* sp. has a discharge papilla flush with the sporangium wall.

Rhizidium richmondense is the host which is most heavily attacked, and all

stages are parasitized. Even the rhizoids of the host are frequently attacked (Fig. 5*l*).

In the development of *Chytridium parasiticum* the zoospore comes to rest on the host and encysts (Fig. 5*a*, *b*). The oscillating granule (see below) remains in motion for some time in the encysted zoospore, and is often a convenient guide for the recognition of this chytrid at this stage. Further development involves unequal expansion of the cyst, the upper portion forming most of the sporangium (Fig. 5*c*, *d*), whilst the lower portion forms the narrow base only. When the sporangium reaches adult size, and before the delimitation of the oil globules of the zoospores, a large area of clear cytoplasm is usually seen at the base of the sporangium. (Fig. 6*c*, *d*). At maturity the sporangium has no obvious papilla, nor is the position of the operculum obvious. The sporangium is pyriform and 8–27μ long by 6–22μ wide. The basal cyst is 2–3μ across. The sporangium may be sessile, or an extramatrical stalk up to 10μ long may attach it to the host. The rhizoidal system of *Chytridium parasiticum* is difficult to demonstrate in living material, but staining with cotton blue has shown that it is delicately branched inside the host (Figs. 5*i*, 6*a*, *b*).

The zoospores in the mature sporangium undergo slight movement before dehiscence takes place. They appear to be 'testing' the wall in the region where dehiscence will occur, as oil globules are seen tightly pressed against the wall in this region. Dehiscence is of the operculate type and extremely violent, tearing of the sporangium wall taking place as the zoospores emerge terminally or laterally. A somewhat similar process has been described by Koch (1951) in the dehiscence of *C. sexuale*. Most of the zoospores are forced out of the sporangium immediately and come to rest some distance from it. They remain quiescent for some moments before moving off. Amoeboid movement of the zoospore body occurs occasionally. The flagellum is posterior and the oil globule anterior; but in addition there is an oscillating granule in the posterior cytoplasm. Zoospores are spherical, 2·2–3·0μ in diameter, average 2·5μ; with an oil globule approximately 1·0μ in diameter, and a flagellum 12–14·6μ long.

The sporangium usually collapses immediately after dehiscence, even before the last of the remaining zoospores have escaped.

C. parasiticum frequently occurs as a parasite on *C. suburceolatum*, which is itself parasitizing *Rhizidium richmondense* (Fig. 7). Less frequently *Chytridium parasiticum* attacks sporangia of *Septosperma rhizophidii* Whiffen (Fig. 9*a*, *c*, *d*). There appears to be no previous record of a hyperparasitic chytrid attacking a chytrid which is itself parasitic on a saprophytic chytrid.

Chytridium parasiticum shows some resemblance to *C. rhizophydii* (Karling, 1948), especially in its extramatrical stalk, delicate rhizoidal system and violent discharge mechanism. The shape of the sporangium and of the operculum, however, is somewhat different in the two species. *C. rhizophydii* also parasitized a chytrid, *Rhizophydium nodulosum*, but seems to be limited to a single host as it failed to attack several other chytrids in the same cultures, none of which are hosts for my fungus. Karling considers that the epibiotic operculate sporangium and endobiotic system of rhizoids place his species in *Chytriomyces* or *Chytridium*. However, since the resting spores are unknown the precise generic position is uncertain. He points

out that most species of *Chytridium* are apophysate and form intramatrical resting spores; whereas in species of *Chytriomyces*, whether the apophysis is present or not the resting spore is extramatrical. Since *Chytridium rhizophydii* is without an apophysis it is unlikely that the resting spores, if found, will prove to be endobiotic. If the resting spores are epibiotic, *Chytridium* would not appear to be an appropriate genus; nevertheless, he places his fungus in that genus rather than in *Chytriomyces*. His reasons for this are that collective swarming of the zoospores, which is characteristic of *Chytriomyces*, does not occur in *Chytridium rhizophydii*; and also that other similar species where the resting spore is not known are placed in *Chytridium*. Karling (1949*a*) has now enlarged the generic conception of *Chytriomyces* by placing in that genus *C. lucidus*, a species in which zoospore swarming does not occur. However, I prefer to follow precedent and name my species as a *Chytridium*. If it should turn out that in either *C. rhizophydii* or *C. parasiticum* an epibiotic resting spore is formed by the conjugation of two thalli, then quite a different genus would be indicated, namely *Zygorhizidium*.

Because of the apparent differences in the infection range of my chytrid and *Chytridium rhizophydii*, and because of minor but seemingly consistent differences in shape, it appears to me that the two cannot be placed in the same species. My species, however, does seem morphologically almost identical with *C. surirellae* (Friedmann, 1953). This, however, is a parasite of the diatom *Surirella* and the emphatic difference in hosts seems to preclude placing my fungus in that species.

Chytridium parasiticum sp.nov. (Figs. 5–7)

Thallus monocentricus, eucarpicus, consistens ex pyriformi sporangio cum angusta basi formata ex inferiore parte cysti zoosporae. Sporangium 8–27 μ longum × 6–22 μ latum, cum terminali aut laterali operculo. Sporangium sessile aut cum extramatricali caule ad 10 μ longo. Systema intramatricale ex rhizoidibus subtiliter ramosum. Zoosporae globosae, 2·2–3 μ diam., cum anteriore guttula olei, 1 μ diam., et flagello posteriore 12–14·6 μ. Restantes sporae non observatae sunt. *Hab.* in vivente *Rhizidio richmondensi* Willoughby, *Rhizophlyctide* sp., *Entophlyctide* sp., *Chytridio suburceolato* Willoughby, et *Septospermate rhizophidii* Whiffen: ex humo in Richmond Park, Surrey, Anglia. Typus: Herb. I.M.I. 60444.

Much of the foregoing discussion concerning the generic position of *Chytridium parasiticum* also applies to *C. suburceolatum*. In this species there is again an epibiotic operculate sporangium and a delicate endobiotic rhizoidal system. Again resting spores were not observed, and the precise generic disposition of this fungus is difficult. Again, I prefer to describe my chytrid as a *Chytridium*. As far as existing species of *Chytridium* are concerned *C. olla* Braun resembles it most closely in morphology. Two dehiscence opercula have previously been described in a species of *Chytridium* by Schulz (1923), who, however, failed to see dehiscence.

Septosperma rhizophidii Whiffen.

This species occurs regularly as a parasite on two multipored species of *Rhizophidium* which are saprophytic on grass leaves. The host sporangium is usually attacked at a young stage before it has produced dehiscence

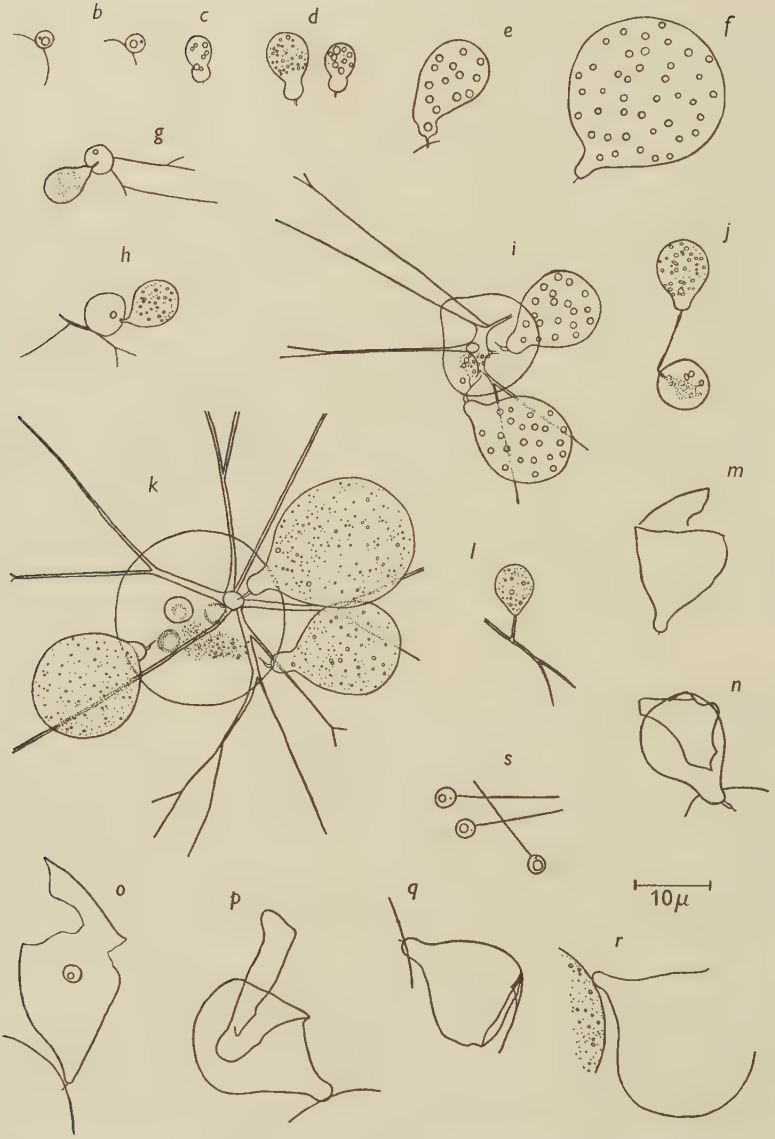

Fig. 5. *Chytridium parasiticum* sp.nov. parasitic on *Rhizidium richmondense*. *a*, *b*, encysted zoospores with oil globule and oscillating granule; *c*, *d*, developing sporangia; *e*, *f*, mature sporangia; *g*, *h*, sporangia on young host thalli; *i*, two mature sporangia on a host sporangium; *j*, sporangium showing long extramatrical stalk; *k*, three immature thalli on a host sporangium the apiculus of which is represented by a circle to the left of its rhizoidal insertion; *l*, sporangium with extramatrical stalk attached to rhizoid of host; *m*–*r*, dehisced sporangia; *s*, zoospores.

papillae which normally never develop. On one occasion, however, abortive dehiscence did occur in a host sporangium bearing thalli of *Septosperma rhizophidii*. This parasite is also found occasionally on *Rhizidium richmondense* and *Rhizophlyctis* sp. In general my material agrees very well

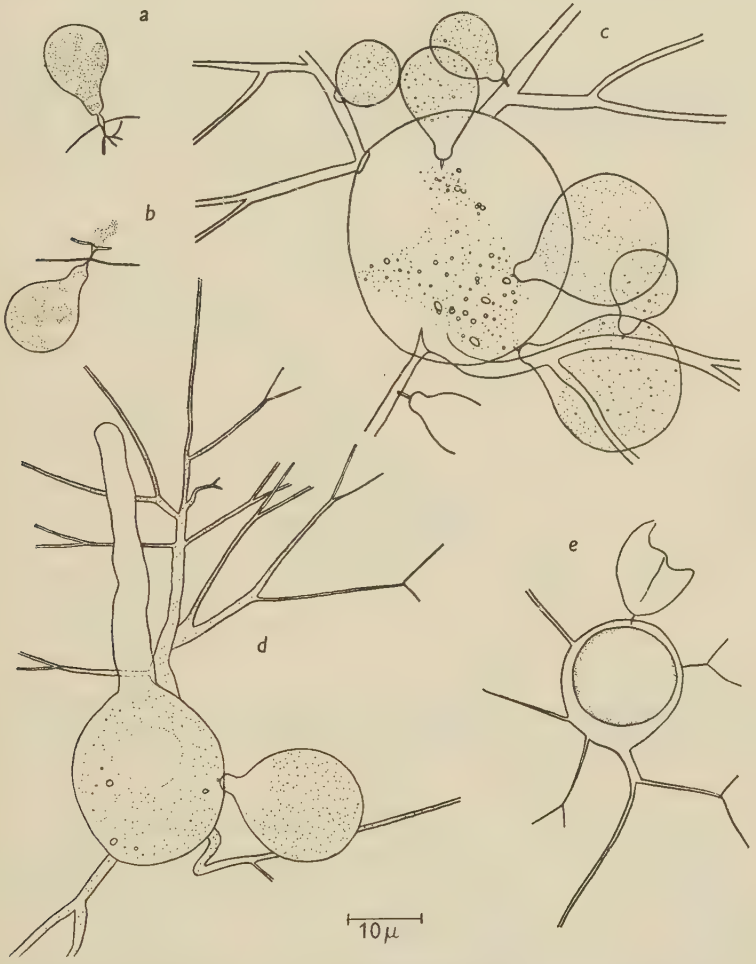

Fig. 6. *Chytridium parasiticum* sp.nov. *a*, *b*, sporangia stained with cotton-blue to show rhizoidal system, on *Rhizophlyctis* sp.; *c*, seven sporangia on a host thallus of *Rhizophlyctis* sp.; *d*, sporangium on a thallus of *Entophlyctis* sp., (the exit tube of the host is figured); *e*, dehisced sporangium on *Rhizophlyctis* sp., the contents of which have retracted to a central oil globule.

with the original description by Whiffen (1942), who found the species on *Rhizophidium macrosporum*. The only other record is that of Sparrow (1952) who reported *Septosperma rhizophidii* as a parasite on cysts of a microscopic animal.

In early stages of attack young thalli can usually be seen clustered on the host in great abundance (Fig. 8*d*). Until they exceed 4μ in diameter they

contain one to three large refractive globules. Subsequent development then determines whether they will become sporangia or resting spores. The former at maturity are spherical or nearly so, and contain a number of evenly spaced oil drops 0·8μ in diameter in clear cytoplasm. Mature sporangia are 10·5–19μ in diameter. Dehiscence is terminal or lateral and involves a dissolution of part of the sporangium wall. There is no discharge papilla. The actual process of dehiscence has only been observed once when it was lateral. In this case the zoospores were 3μ in diameter with a posterior flagellum and a conspicuous oil drop in clear cytoplasm.

Young resting spores are recognized by their elongated shape. The distal end comes to contain a number of oil globules, and the proximal end

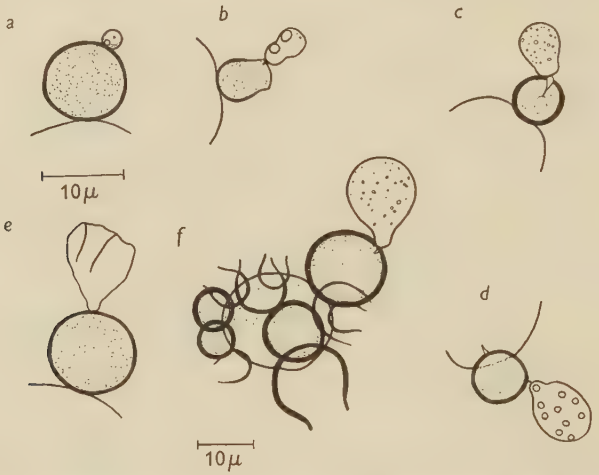

Fig. 7. *Chytridium parasiticum* sp.nov. hyperparasitic on *C. suburceolatum*, which is itself parasitic on *Rhizidium richmondense*. *a*, encysted zoospore with oil globule and oscillating granule; *b*, *c*, developing sporangia; *d*, mature sporangium; *e*, dehisced sporangium; *f*, young sporangium top right. All drawings at same scale except *f*.

becomes vacuolated (Fig. 8*d*, *e*) and eventually a cross-wall forms cutting off this portion, which by this time is completely empty. In the fully matured condition the oil globules in the distal cell are minute and a clear area has been seen in the middle of this, 2·5μ in diameter. This possibly represents the position of the nucleus.

The rhizoidal system of young thalli takes the form of a sac-like haustorium (Fig. 8*a*, *e*, *i*). Immature and empty sporangia and resting spores are, however, usually observed to have a bifurcated rhizoidal system (Fig. 8*e*, *g*, *j*). In some maturing resting spores a sac-like haustorium is present (Figs. 8*b*, *i*, 9*c*), with contents of the same texture as the host cytoplasm. This suggests that the rhizoidal system is either sac-like in all cases, appearing bifurcated only because its complete outline is partially obscured; or that the sac collapses in older thalli to give the appearance of a bifurcated rhizoid. A branching into three rhizoidal axes has never been seen. Mature resting spores are 10·5–21μ long by 5–8μ wide. Resting spore germination has not been observed.

Septosperma rhizophidii also parasitizes *Rhizidium richmondense* (Fig. 9*e*) and *Rhizophlyctis* sp. (Fig. 9*a*, *b*), the host occurring on onion scale epidermal strippings. The hyperparasitic attack of *Septosperma rhizophidii* by *Chytridium parasiticum* has already been discussed.

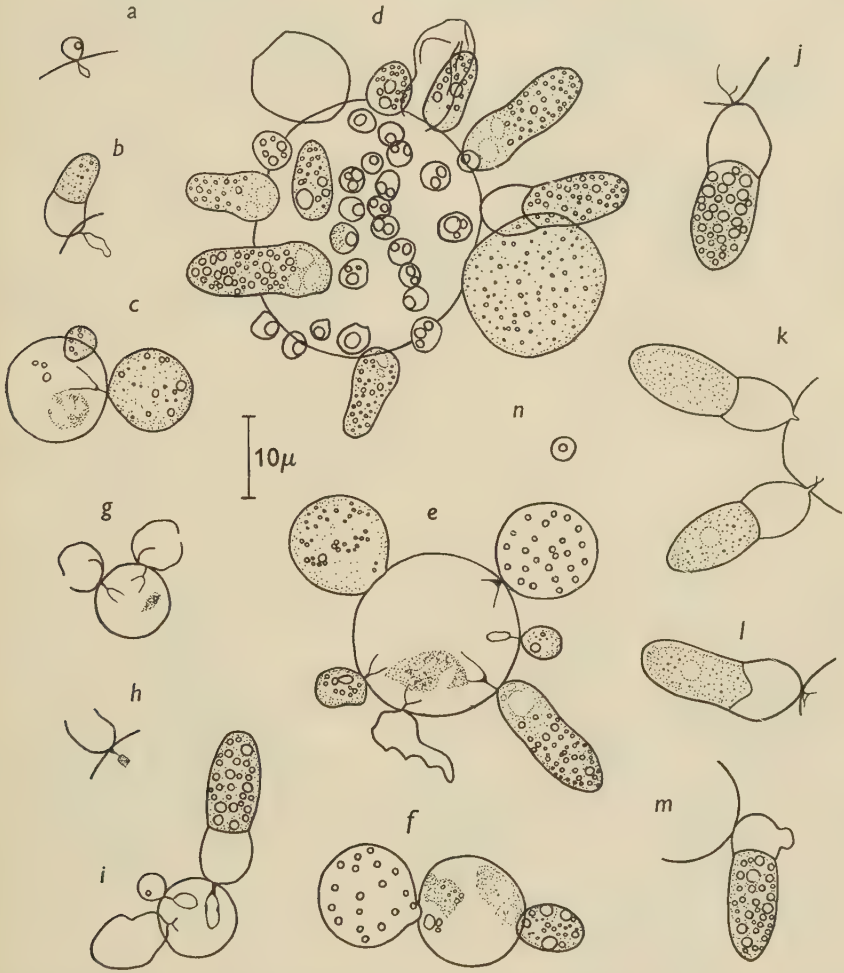

Fig. 8. *Septosperma rhizophidii* Whiffen parasitic on multipored *Rhizophidium* sp. *a*, young thallus with sac-like haustorium; *b*, resting spore with sac-like haustorium; *c*, *d*, sporangia and resting spores in various stages of development on host thalli; *e*, six parasitic thalli on a host thallus (a mature sporangium is at top right and a developing resting spore showing vacuolation in the proximal end at bottom right); *f*, two sporangia on a host thallus, the one on the left at maturity; *g*, *h*, dehisced sporangia; *i*, three thalli on a host thallus, the resting spore right with a sac-like haustorium; *j*, resting spore with bifurcated rhizoidal system; *k*, *l*, mature resting spores with central clear area in the distal cell; *m*, resting spore; *n*, zoospore.

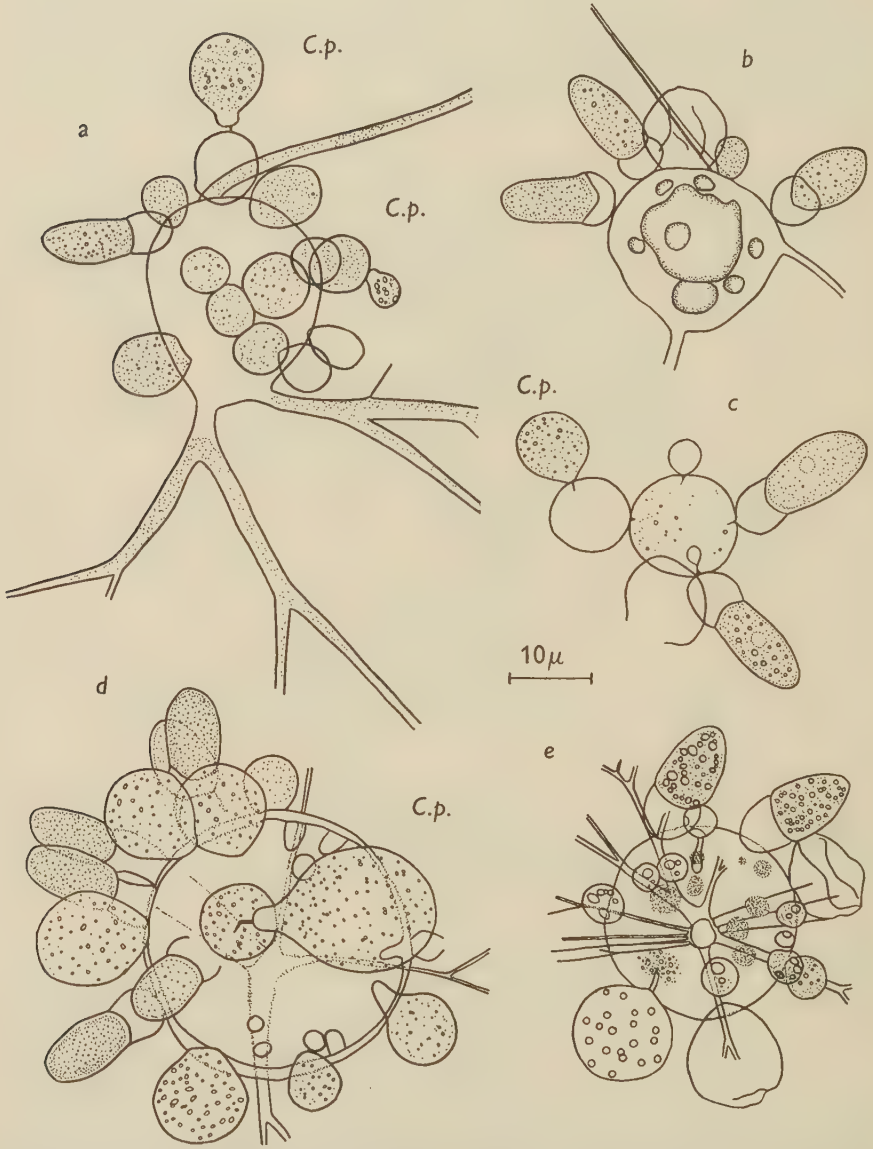

Fig. 9. *a*, *b*, thalli of *Rhizophlyctis* sp. bearing sporangia and resting spores of *Septosperma rhizophidii*; *c–e*, thalli of *Rhizidium richmondense* bearing sporangia and resting spores of *S. rhizophidii*. In *d*, solid plugs are attached to the host sporangium wall in various places. In two cases these plugs are surmounted by sporangia of the parasite, and they are presumably formed by the host as the result of parasitic attack. In *a*, *c*, *d*, *Chytridium parasiticum* is hyperparasitizing sporangia of *S. rhizophidii*. Hyperparasitic sporangia are labelled *C.p.*

Discussion

Fairly intensive work on soil from one site indicates that a regular and constant flora of chytrids is present. The same species are obtained again and again throughout the year on the various kinds of baits used. However, the soil chytrids described in this paper form only a part of the chytrid flora from the site investigated; and other forms which are less completely known will be considered later. Preliminary investigations at other places have yielded many of the same organisms; suggesting that they are widely distributed, as far as the London area is concerned.

My thanks are due to Prof. C. T. Ingold for the help and advice he has given throughout the course of this work, and to Prof. E. H. Warmington for the Latin diagnoses. I should also like to thank Dr Hilda Lund for past encouragement. Most of the work has been carried out with the aid of a maintenance grant from the Department of Scientific and Industrial Research, to which acknowledgement is made.

REFERENCES

Bartsch, A. F. (1939). Reclassification of *Chytridium spinulosum* with additional observations on its life history. *Mycologia*, **31**, 558–571.

Couch, J. N. (1939). Technic for collection, isolation and culture of Chytrids. *J. Elisha Mitchell sci. Soc.* **55**, 208–214.

Fay, D. J. (1947). *Chytriomyces spinosus* nov.sp. *Mycologia*, **39**, 152–157.

Friedmann, I. (1953). Eine neue Chytridiale, *Chytridium surirellae* n.sp. *Öst. bot. Z.* Bd. 100, H. 1/2, 5–7.

Hanson, A. M. (1945). A morphological, developmental, and cytological study of four saprophytic Chytrids. I. *Catenomyces persicinus* Hanson. *Amer. J. Bot.* **32**, 431–438.

Haskins, R. H. (1939). Cellulose as a substratum for saprophytic Chytrids. *Amer. J. Bot.* **26**, 635–639.

Karling, J. S. (1946). Keratinophilic Chytrids. I. *Rhizophydium keratinophilum* n.sp., a saprophyte isolated on human hair, and its parasite, *Phlyctidium mycetophagum* n.sp. *Amer. J. Bot.* **33**, 751–757.

Karling, J. S. (1948). Keratinophilic Chytrids. III. *Rhizophydium nodulosum* sp.nov. *Mycologia*, **40**, 328–335.

Karling, J. S. (1949). Two new eucarpic inoperculate Chytrids from Maryland. *Amer. J. Bot.* **36**, 681–687.

Karling, J. S. (1949*a*). Three new species of *Chytriomyces* from Maryland. *Bull. Torrey bot. Cl.* **76**, 352–363.

Koch, W. J. (1951). Studies in the genus *Chytridium*, with observations on a sexually reproducing species. *J. Elisha Mitchell sci. Soc.* **67**, 267–278.

Schulz, P. (1923). Kurze Mitteilungen über Algenparasiten. *Schriften f. Süsswasser und Meereskunde*, 2 (11), 178–181.

Sparrow, F. K. (1952). Phycomycetes from the Douglas Lake region of northern Michigan. *Mycologia*, **44**, 759–772.

Whiffen, A. J. (1942). Two new Chytrid genera. *Mycologia*, **34**, 543–557.

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Trans. Brit. mycol. Soc. **39** (1), 142–144 (1956).

REVIEWS

Die Gattungen der amerosporen Pyrenomyceten. By J. A. von Arx and Emil Müller. (Beiträge zur Kryptogamenflora der Schweiz, Band 11, Heft 1. Bern: Büchler & Co. 1954). 434 pp., 119 figures. Price 30 Swiss francs.

Developmental studies of Pyrenomycetes during the past thirty years have emphasized the unsatisfactory nature of earlier systems of classification, and have prompted various authors, notably Nannfeldt, Miller, Luttrell and Munk, to propose more natural groupings. These attempts have in turn revealed inconsistency at the generic level, and have made urgent the task of critical re-examination of genera in order to determine their positions in a rational classification. The authors of this work have provided their own version of a natural classification, and have at the same time presented the results of their extensive re-examination of herbarium material. The magnitude of this task has compelled a restriction of effort to the amerosporous pyrenomycetes, excluding the Erysiphales and forms with allantoid spores. It is to be hoped that the authors will be encouraged to give attention to the remaining spore-forms.

The system of classification emphasizes the distinction between forms with bitunicate asci (Bitunicatae or Ascoloculares) and those with unitunicate asci. The Bitunicatae are divided into the Myriangiales, Dothiorales and Pseudosphaeriales. Since only the Dothiorales contains genera with unicellular spores, only this group is considered further. The amerosporous forms are contained in three families; Entopeltaceae, Botryosphaeriaceae and Mesnieraceae. The Hysteriaceae is also classified here, but contains only multicellular forms. The Unitunicatae are distributed within four orders, Phacidiales, Sphaeriales, Diaporthales and Clavicipitales. Three families, Cryptomycetaceae, Phacidiaceae and Hypodermataceae, are recognized within the Phacidiales, but only the first two receive detailed treatment. The Sphaeriales are re-defined to exclude heterogeneous elements included by Lindau, and now include the Melanosporaceae for *Melanospora*, *Chaetomium* and related genera, the Polystigmataceae for *Glomerella*, *Polystigma*, *Coccostroma*, *Phyllachora* etc., and the Xylariaceae defined in a broader sense than usual to include such genera as *Bombardia*, *Lasiosordaria*, *Sordaria*, *Neurospora* and *Neocosmospora*. *Ustulina* is included in *Hypoxylon* and *Anthostomella* in *Anthostoma*. The Diaporthales are distinguished from the Sphaeriales by a different type of apical apparatus in the ascus and by the arrangement of the fructifications. There are two families, the Diaporthaceae and the Valsaceae, but the latter are arbitrarily excluded from further consideration on account of their allantoid spores.

An extensive synonymy results in part from a revision of specimens, but in part from a study of descriptions only. The treatment has in general been broad, so that *Botryosphaeria quercuum* and *Glomerella cingulata* each have a list of over 100 synonyms. It can be argued that a general morphological similarity between species is not enough to warrant such 'lumping', and that culture studies, including comparison of conidial states and host specificity would be necessary to support such treatment. In certain genera re-examination of the type species results in a more precise circumscription of the genus. Thus *Physalospora* is reduced to nine species. An annotated list follows giving the authors' views on the disposition of about 150 names earlier described in the genus. Many of these names are rejected without adequate reason.

Keys to families and genera are given, and excellent figures for many genera which convey clearly the structure of the perithecia and stromata. An annotated list of doubtful genera is appended.

Criticism may perhaps be made of inconsistent treatment. In some orders a complete key to families is given, but in others only the amerosporous families are keyed. Whilst some genera are monographed, others have only the type species described. It is also regrettable that some of the less well-known genera are not figured. Further criticism can be made of the choice of names for certain taxa. The citation of numerous new combinations is difficult to locate, and a list of new taxa would have been a useful addition.

Such criticisms do not, however, seriously detract from the value of this much-needed revision. Indeed the scope of the undertaking is such that few would have been capable of it. Detailed criticism will doubtless follow as specialists assimilate its contents, but this volume marks an important step forward in the progress towards a satisfactory classification of Pyrenomycetes.

J. WEBSTER

Die Röhrlinge, Blätter- und Bauchpilze (Agaricales und Gasteromycetales). By MEINHARDT MOSER. (Kleine Kryptogamenflora. Basidiomyceten II. Stuttgart: Gustav Fischer Verlag. 1955.) 2nd edition. 327 pp., 17 text-figures. Price: DM. 16.50.

Perhaps the most useful feature of this second edition is the treatment of *Cortinarius*. The author's extensive investigations of this genus have enabled him to present a fresh treatment of the genera *Phlegmacium* (Fr.) Fayod and *Hydrocybe* (Fr.) Wünsche. Whilst the incorporation of parts of the Friesian subgenera *Inoloma* and *Dermocybe* as a subgenus of *Phlegmacium* has yet to prove its value, the rearrangement of the species of the Friesian subgenus *Phlegmacium* with abolition of the rather overlapping Friesian sections: Scauri, Cliduchi and Elastici is undoubtedly helpful. The division of *Hydrocybe* into groups separated by stem characters is less revolutionary but is probably as useful an approach as any in this very involved and highly critical group, almost impossible to treat satisfactorily without more detailed descriptions.

Taken as a whole the book is likely to be more useful as a pocket 'aide memoire' to those who already have some knowledge of toadstools than as a book for beginners. It is perhaps unfortunate that in many cases no mention is made of spore shape (often critical) —the one or two extra words required would surely not have increased the size of the book appreciably—and it would also have been useful, when size of cap is used as a key character, to have used another character in addition, since size of cap alone is often liable to be misleading. It is very much to be hoped that the names of the several new species included will very soon be validated elsewhere. The nomenclature used is that introduced by Singer in *The Agaricales in Modern Taxonomy* (1949), which seems likely to be adopted in the main by most countries, so that this book serves as a useful introduction to those who are unfamiliar with it. The better quality paper and layout, the numbering of the species and especially the excellent and attractive durable binding are a great improvement on those of the first edition.

P. D. ORTON

I Funghi. Vita, Storia, Leggende. By L. MORANDI and E. BALDACCI. (Milan: Garzanti. 1954.) viii+633 pp., 43 plates (32 in colour), 26 text-figures. Price 4500 lire.

This book invites comparison with Ramsbottom's *Mushrooms and Toadstools*, for the two works, though owing nothing to one another, have much in common. They provide yet another example of the effects of the 'climate of opinion' on mycological authors. Until recently there was no text-book on the physiology of fungi, then two appeared within a year. Ramsbottom's unique '20th century Anatomy of Toadstools' (according to the dust jacket) appeared in 1953 and in 1954 this Italian counterpart. English readers who have savoured Ramsbottom's book will have some idea of the scope of that by the mycologist Baldacci and the bibliophile Morandi with its blend of science, history, bibliography and legend.

The first half of the book includes chapters on the classification and nature of fungi, their chemical composition, physiology and uses together with accounts of edible and poisonous forms, and the various indexes to the volume are preceded by a useful 16-page chronologically arranged and annotated bibliography from Aristotle to the end of the nineteenth century.

The second half is primarily a semi-popular account of the commoner larger fungi of Italy with various tabulations and keys to assist with their identification. The arrangement of the descriptions is very unusual, for there is a primary division into edible and inedible species and descriptions of the former occupy successive left-hand pages, those of

the latter the right-hand pages. As approximately 70% of the species described are edible, the right-hand pages also contain much supplementary, and often curious, information on the genera under consideration. In addition to a number of black and white text-figures and plates there are 16 pages of coloured plates on which more than 140 species of larger fungi are attractively but somewhat impressionistically represented.

The book can be commended to all those interested in mycological byways.

G. C. AINSWORTH

An Introduction to Botany. By J. H. PRIESTLEY and LORNA I. SCOTT. 3rd edition (London: Longmans, Green and Co. 1955). 627 pp., 169 text-figures. Price 30*s.*

This well-known and much appreciated Introduction to Botany can rarely have fallen into the hands of a mycologist for review. Such a reviewer is warned on the title-page that the text has 'special reference to the structure of the flowering plant' and on inspection of the book he finds that only one of the thirty-nine chapters is devoted to fungi. This deals with 'fungi as parasites and saprophytes' and appears to have been inserted to introduce the traditional elementary types, *Puccinia*, *Mucor*, and *Saccharomyces*. Space forbids a consideration of the status of mycology in a botanical course and comment must be limited to noting that the atypical *Puccinia graminis* is not the most happy choice as an introduction to fungi, especially in a work giving emphasis to higher plants. The very existence of higher plants is dependent on the saprophytic activities of fungi and bacteria and it is this aspect which needs stressing if a proper perspective is to be attained. The chapter on fungi appears to be unchanged from the previous edition. For the next it might well be recast.

G. C. AINSWORTH

Erkenne und Bekampfe den Hausschwamm und seine Begleiter! By KURT LOWAG. (Vienna: Verlag Georg Fromme and Co. 1955) 61 pp., 9 coloured and 36 uncoloured figures. Price: DM. 8.00.

This attractively produced booklet gives concise, semi-popular, well-illustrated accounts of nineteen timber destroying fungi together with indications of the damage they cause. In conclusion there is a bibliography of thirty-two references and an index to common and scientific fungal names and synonyms.

The Genus Cortinarius. *I.* Myxacium *and* Phlegmacium. By P. D. ORTON. 80 pp., 17 figures. (Reprinted Supplement to *The Naturalist*, July–September, 1955). Price 7*s.* 6*d.* (From the editor, *The Naturalist*, The University, Leeds, 2.)

'This paper is founded on notes left by the late A. A. Pearson, F.L.S., now in the Herbarium of the Royal Botanic Gardens at Kew... completely revised and extended... to include many details and some extra species' by P. D. Orton. It is on similar lines to earlier issues of the series and includes a key to 102 species for which the characteristics are fully and skilfully presented in tabular form. Two further parts, on the subgenera *Inoloma* and *Dermocybe* and *Telamonia* and *Hydrocybe*, are promised.

Rusts of British Forest Trees. By J. S. MURRAY. 15 pp., 13 figures (9 in colour). (Forestry Commission Booklet No. 4. London: H.M.S.O. 1955.) Price 2*s.* 6*d.*

Gives brief non-technical accounts of the symptoms caused by nearly twenty rusts which affect forest trees in the British Isles, together with indications of the alternate hosts, the economic importance, and recommendations for control.